ENHANCING MAIZE (*Zea mays* L.) YIELD AND Zn CONTENT WITH Zn APPLICATION THROUGH SEED, SOIL AND FOLIAR METHODS

ABSTRACT

During *Kharif* 2022, an experiment was conducted at Student's Research Farm, Department of Agronomy, Khalsa College, Amritsar, Punjab by using a split plot design with three replications of each of the 16 treatments. In comparison to control, main plots with seed inoculation with *Bacillus subtilis* and soil application of ZnSO4 @ 16.25 kg ha^{-1} (S_3) showed significantly greater yield and quality parameters. In case of sub plot treatments, similar results were seen with foliar spray of $ZnSO_4$ at 45 + 75 DAS (F_3).

Keywords: Foliar application, Maize, Seed application, Soil application, Zn.

1. INTRODUCTION

Zn (Zn) is one of the most important micronutrients that is needed in plant tissues in contents ranging from 5 to 100 mg kg^{-1} for optimum growth and development (Ghosh *et al*., 2019). The performance and quality of crops can be impacted by Zn deficiency since it can limit growth, stress tolerance, photosynthesis, RNA synthesis, and protein synthesis. Malnutrition from Zn affects a large number of people globally. According to the FAO (2014), one-third of the world's population is at high risk of Zn deficiency. In a survey by the World Health Organization, Zn deficiency is ranked fifth among the most significant health risk factors in developing nations and eleventh overall. Zn deficiency in people impairs the immune system, makes people more susceptible to infection, leads to diarrhea, poor wound healing, hair loss, unexplained weight loss, depression, increased anxiety, emotional instability, problems with learning and memory, seizures, and has an impact on both the physical development of children and pregnancy in women (Mumtaz *et al*., 2020). People who have lower blood Zn levels than average are more likely to develop certain disorders, such as Alzheimer's and Parkinson's (Brewer *et al*., 2010). Animals with Zn deficiencies exhibit lethargy, alopecia in different body parts, skin parakeratosis, stunted growth, swollen joints and decreased milk supply. One of the most vulnerable cereal crops to a Zn deficiency is maize. A number of agronomic, soil, and environmental factors are thought to be responsible for the estimated 50% Zn deficiency of the world's soils (Alloway, 2009). Zn deficiency in soils is caused by inadequate addition of organic matter in the soil, intensive farming practices that remove a significant quantity of minerals from the soil and insufficient use of micronutrient fertilizers. According to several studies (Hossain et al., 2008; Potarzycki and Grzebisz, 2009; Harris et al., 2007), it is reported that applying Zn increases maize grain output globally. Farmers recognized maize as having the highest yield in relation to Zn supply (Leach and Hameleers, 2011; Subedi and Ma, 2009). Application of Zn is another successful biofortification tactic. With biofortification, Zn is intended to be added to common foods like rice, wheat, maize, pearl millet, and others. It is accomplished through the direct administration of micronutrients to crop foliage, soil application and/or seed treatment. Foliar fertilizer application is a more efficient, cost-effective and fertilizer use efficient strategy that also reduces losses of nutrients and environmental degradation. While foliar spray promotes Zn accumulation in grains, applying Zn to the soil also works well to increase grain yield (Ehsanullah et al., 2015). Maize can also be biofortified by seed treatment with Zn-solubilizing bacteria (ZSB), such as *Bacillus* spp. In order to increase crop yield, growth and nutrient absorption in grains, *Bacillus* spp. are also most prevalent. They promote Zn uptake and accumulation in grains by converting inaccessible forms of Zn into forms that plants can use. Although Zn can be applied in a number of ways, including through seed, soil, and foliar treatment, we need to identify the most efficient way to do so. The current study was created with the aforementioned factors in mind in order to assess the most efficient way to raise the Zn content in maize grains as well as to examine the impact of different Zn application methods on the yield of the maize crop.

2. MATERIALS AND METHODS

The current experiment was carried out in the Student's Research Farm, Department of Agronomy at the Khalsa College in Amritsar, Punjab, during *Kharif* 2022 to investigate the impact of Zn on quality and production of maize crop. The experimental site was located at 31.63 ^{0}N and 74.83 ^{0}E with a height of 234 m above sea level. Rating limits for different soil characteristics are explained in table 1. The experimental soil in the study region was a sandy loam with a normal pH of 8.3, a normal EC of 0.21 dS m^{-1}, a low organic carbon content of 0.38%, low available nitrogen of 163.2 kg ha^{-1}, medium available phosphorus of 18 kg ha^{-1}, medium available potassium of 263 kg ha^{-1} and deficient available Zn of 0.44 mg kg^{-1}.

Table 1. Rating limits for different soil characteristics

Soil characteristics	Categories		
pH	Normal (6.5-8.7)	Marginally alkaline (8.7-9.3)	Alkali or sodic (> *9*.3)
Electrical conductivity (dS m^{-1})	Normal (< 0.8)	Critical for crop production (>0.8)	
Organic carbon (%)	Low (< 0.40)	Medium (0.40-0.75)	High (>0.75)
Available N (kg ha^{-1})	Low (< 271)	Medium (271-543)	High (>543)
Available P (kg ha^{-1})	Low (< 12)	Medium (12-22)	High (>22)
Available K (kg ha^{-1})	Low (< 136)	Medium (136-333)	High (>333)
DTPA extractable Zn (mg kg^{-1})	Deficient (< 0.6)	Sufficient (>0.6)	

A split plot design was used for the experiment, which was replicated thrice. The experiment included 16 treatments *viz.,* four main plot treatments {control (S_0), seed inoculation with *Bacillus subtilis* (S_1), soil application of $ZnSO_4$ @16.25 kg ha^{-1} (S_2), seed inoculation with *Bacillus subtilis* + soil application of $ZnSO_4$ @16.25 kg ha^{-1} (S_3)} and four sub plot treatments *viz.,* {control (F_1), foliar spray @0.5% $ZnSO_4$ at 45 DAS (F_1), foliar spray @0.5% $ZnSO_4$ at 75 DAS (F_2), foliar spray @0.5% $ZnSO_4$ at 45 + 75 DAS (F_3)}. Urea was used to provide nitrogen in three separate doses. Zn fertilizer (Zn sulphate monohydrate) was applied to the soil together with one-third of the nitrogen during sowing. At the knee-high stage and pre-tasseling stage, the remaining nitrogen dose was distributed equally. Zn solubilizing bacteria (ZSB) was used to inoculate seeds prior to sowing, after which the seeds were shade dried. Sowing was done on ridges with the help of bed planter. Rows were spaced 60 cm apart and plants were spaced 20 cm apart during the sowing process. Foliar applications of $ZnSO_4$ were applied at 45 and 75 DAS. The observations were made at 25, 50, and 75 DAS and at harvest. Under the experimental trial, yield data including grain yield (q ha^{-1}), straw yield (q ha^{-1}) and quality parameters including Zn content (mg kg^{-1}) and Zn uptake (g ha^{-1}) in grain and stover were observed. Zn content in grain and stover was determined by using micro plasma atomic emission spectrophotometer (MPAES). In order to determine Zn content in grain and stover, 1 g of processed grain and stover sample was taken in 250 ml Erlenmeyer flask and 15-20 ml of di-acid mixture was added to it. It was heated on a hot plate. The sample was allowed to further heat until the contents of the flask gave a yellowish green appearance and volume was reduced to about 3-5 ml but not to dryness, indicating completion of digestion. After cooling the contents in the flask, digested material was transferred into a 100 ml volumetric flask and made volume 100 ml with distilled water. Aliquot of this solution was used to analyze Zn in plant samples with the help of (MPAES). The following formula was used to calculate the Zn uptake in grains and stover:

$$\text{Zn uptake in grain (g ha}^{-1}\text{)} = \frac{\text{Zn content of grain (mg kg}^{-1}\text{)} \cdot \text{Grain yield (kg ha}^{-1}\text{)}}{1000}$$

$$\text{Zn uptake in stover} = \text{(g ha}^{-1}\text{)} \quad \frac{\text{Zn content of stover (mg kg}^{-1}\text{)} \cdot \text{Stover yield (kg ha}^{-1}\text{)}}{1000}$$

3. RESULTS AND DISCUSSION

3.1 Yield parameters

3.1.1 Grain yield (q ha^{-1})

A crop's grain yield is the result of all the management and other factors that affect growth and yield attributing characteristics throughout the crop's life cycle. The final impact of various treatments on crop economic yield can be used to assess their true efficacy. Table 2 shows the yield information of maize grain. The combination of seed inoculation (*Bacillus subtilis*) + soil application of $ZnSO_4$ @16.25 kg ha^{-1} (S_3) produced the highest grain yield (57.5 q ha^{-1}) of maize and was significantly higher than control (S_0), seed inoculation (*Bacillus subtilis*) (S_1) but at par with soil application of $ZnSO_4$ @16.25 kg ha^{-1} (S_2). Treatment S_2 was significantly higher than S_0 but at par with S_1. The grain yield of S_3 was 3.79, 10.58, and 16.16 % higher than that of S_2, S_1, and S_0, respectively. According to Singh *et al*. (2019), Zn soil application during sowing performed noticeably better than control because application of Zn increased the availability of carbohydrates to kernels, increasing yield components such as cob length, number of grains cob^{-1} and test weight. Improvement in vegetative development of the crop plant produced direct impact on grain output. Foliar treatments had a considerable impact on grain yield as well. Significantly higher grain yield was recorded under foliar spray @0.5% $ZnSO_4$ at 45 + 75 DAS (F_3) than control (F_0) and foliar spray @0.5% $ZnSO_4$ at 75 DAS (F_2) but was closely followed by foliar spray @0.5% $ZnSO_4$ at 45 DAS (F_1). The treatment F_1 was statistically at par with F_2 but showed significantly better results over F_0. F_3 caused up to 1.27, 6.46, 10.89 % increase in grain yield compared to F_1, F_2 and F_0 respectively. According to Jadhav *et al*. (2019), early vegetative stages of plants treated with $ZnSO_4$ spray produced higher grain yield than control. He observed that foliar nutrition enhanced nutrient uptake and translocation by maize plants which resulted in a notable increase in grain output. The synthesis, accumulation and translocation of photosynthates depend upon efficient photosynthetic structure as well as the extent of translocation into sink (grains) and also on plant growth and development during early stages of crop growth. This may be attributed to fulfillment of the demand of the crop by higher assimilation and translocation of photosynthates from source (leaves) to sink (grains) through supply of required nutrients by foliar spray (Jadhav *et al*. (2019).

3.1.2 Stover yield (q ha^{-1})

Stover yield of maize has economic worth as it is fed to the animals. It is an important parameter of the biological yield to evaluate its productivity index for judging the ultimate performance of a crop. Table 2 contain information about the yield of stover. The findings showed that S_3 (seed inoculation with *Bacillus subtilis* + soil

Table 2. Effect of seed, soil and foliar application of Zn on grain yield and stover yield of maize

Treatments	Grain yield (q ha^{-1})	Stover yield (q ha^{-1})
Main plots		
Control	49.5	68.7

Seed inoculation (*Bacillus subtilis*)	52.0	70.9
Soil application of $ZnSO_4$ @16.25 kg ha^{-1}	55.4	74.4
Seed inoculation (*Bacillus subtilis*) + soil application of $ZnSO_4$ @16.25 kg ha^{-1}	57.5	76.4
CD (*P*= 0.05)	3.77	5.26
Sub plots		
Control	50.5	69.4
Foliar spray @0.5% $ZnSO_4$ at 45 DAS	55.3	74.1
Foliar spray @0.5% $ZnSO_4$ at 75 DAS	52.6	71.4
Foliar spray @0.5% $ZnSO_4$ at 45 + 75 DAS	56.0	75.6
CD (*P*= 0.05)	2.77	3.90

Interactions were found to be non-significant.

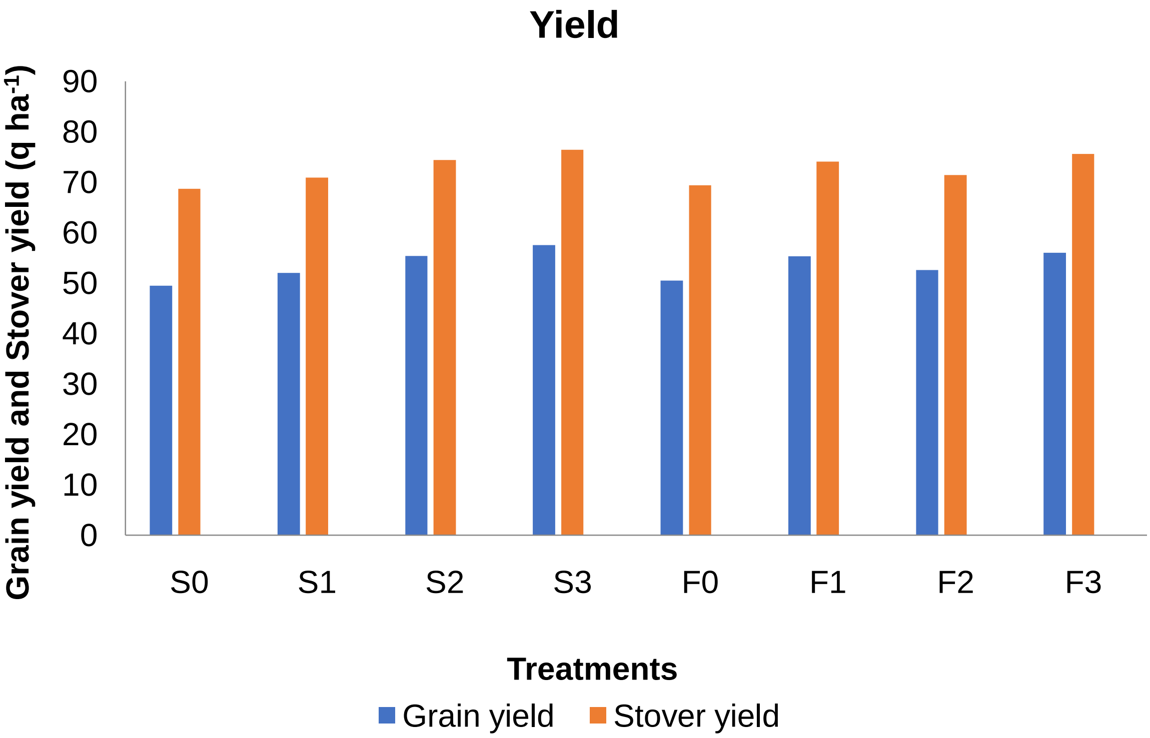

Fig. 1: Effect of seed, soil and foliar application of Zn on grain yield, stover yield of maize

application of $ZnSO_4$ @16.25 kg ha^{-1}) had the highest recorded stover yield of 76.4 q ha^{-1}, which was significantly higher than S_1 (seed inoculation with *Bacillus subtilis*) and S_0 (control) but was at par with S_2 (soil application of $ZnSO_4$ @16.25 kg ha^{-1}). Statistically, the treatment S_2 was at par with S_1 but greatly outperformed S_0 in terms of results. Stover yield for S_3 increased by 11.21, 7.76, and 2.69 % over S_0, S_1, and S_2, respectively. In line with these results, Singh *et al*. (2021) reported soil application of 30 kg ha^{-1} Zn. According to the results of Peddapuli*et al*. (2021), the soil application of RDF + Zn @25 kg ha^{-1} increased stover production compared to the control, which may be because Zn speed up photosynthate translocation from source to sink and consequently enhance the yield of stover. According to the data in the table 2, treatment F_3 (foliar spray @0.5% $ZnSO_4$ at 45 + 75 DAS) had the greatest recorded stover yield followed by treatments F_1 (foliar spray @0.5% $ZnSO_4$ at 45 DAS) and F_2 (foliar spray @0.5% $ZnSO_4$ at 75 DAS) while treatment F_0 (control) had the lowest recorded stover yield. Treatment F_1 was significantly higher than F_0 but at par with F_2. However, significant higher stover yield was recorded under F_3 by 8.93 than F_0 and F_2 by around 5.88 % respectively. According to Gomaa *et al*. (2022), spraying Zn @1.2 kg ha^{-1} at 40 and 50 DAS improved stover yield compared to control. When Zn is applied in sufficient amounts, it aids in the regulation of numerous physiological and metabolic processes. It also promotes better nitrogen accumulation, which increases plant height, dry weight, and ultimately stover output.

3.2 Quality parameters

3.2.1 Zn content in grain (mg kg^{-1})

A potential yield response to applied Zn is shown by the Zn content of maize grains. Grain Zn content was revealed by data in table 3. Data analysis showed that S_3 treatment (seed inoculation (*Bacillus subtilis*) + soil application of ZnSO4 @16.25 kg ha^{-1}) had the highest Zn content in grain (42.59 mg kg^{-1}) whereas S_0 (control) had the lowest (36.41 mg kg^{-1}). In comparison to previous treatments, S_3 revealed considerably more Zn in the grain which was significantly higher than that of S_1 and S_0 but at par with S_2 treatment. The treatment S_2 also gave superior results over S_0 and S_1. Zn content of grain under S_3 treatment increased by 0.78, 14.27 and 16.97 % over S_2, S_1, and S_0, respectively. Similar findings were made by Kandali*et al*. (2021), who discovered that application of Zn to the soil at various quantities increased grain Zn content compared to the control. For foliar application, treatment F_3 treatment (foliar spray @0.5% $ZnSO_4$ at 45 + 75 DAS) had a higher Zn content of grain (43.23 mg kg^{-1}) than control treatment F_0, which had a lower Zn content (36.65 mg kg^{-1}). However, F3 treatment was significantly greater than F_0 and F_2 but at par with F_1 in case of Zn content of grain. However, treatment F_1 was significantly better than F_0 but equivalent to F_2 in terms of effectiveness on Zn content of grain. The Zn content of grain of F_3 increased around 6.61, 13.46, and 17.95 % respectively over F_1, F_2 and F_0. According to Arabhanvi*et al*. (2018), foliar application of $ZnSO_4$ and $FeSO_4$ @0.5% each at 40 DAS increased the Zn content in grain compared to the control. Increase in micronutrient uptake and content is caused by fixation of externally given inorganic Zn and Fe into organically bound and naturally chelated form of Zn and Fe which favoured their availability and hence boosted uptake (Jan *et al*., 2020).

3.2.2 Zn content in stover (mg kg^{-1})

Zn content in stover is presented in table 3. According to the data, the main plots with seed inoculation (*Bacillus subtilis*) + soil treatment of $ZnSO_4$ @16.25 kg ha^{-1}(S_3) had higher Zn content (56.43 mg kg^{-1}) than control (S_0), which had lower Zn content (47.65 mg kg^{-1}).S_2 demonstrated significantly higher Zn content in stover compared to S_0 but at par with S_1. The treatment S_3 was shown to have an increase of 2.81, 7.94, and 18.43 % over S_2, S_1, and S_0, respectively. The outcomes was in line with results of Tariq *et al*. (2014) who observed that Pioneer-32F 10, Monsanto-6525 and Hycorn-8288 preserved the highest Zn contents in stover as compared to control after soil application of Zn. The findings showed that maize uptakes and accumulates more Zn in stover than in the grains. Zn content in stover was considerably impacted by foliar treatments. Under foliar spray @0.5% $ZnSO_4$ at 45 + 75 DAS (F_3), the maximum Zn content in stover (54.87 mg kg^{-1}) was discovered, whereas the lowest Zn content in stover (49.06 mg kg^{-1}) was recorded in the control (F_0). Treatment F_3 showed significantly higher results than F_0 but showed closed results with F_1 and F_2. Foliar application F_1 gave significantly better results over F_0 and gave close results with F_2. The increase in Zn content of stover of F_3 was2.16, 2.35, and 11.84 % compared to F_1, F_2, and F_0, respectively. In addition, Kumar *et al*. (2017) found that Zn content in fodder maize was highest after one foliar application of $ZnSO_4$ at 30 DAS and two foliar applications at 30 & 45 DAS compared to control. This is because of role of Zn in photosynthesis and metabolic process which increases the production of photosynthates and their translocation to different plant parts, which ultimately increases the content of Zn in stover. According to research by Arabhanvi*et al*. (2018), foliar treatment of $ZnSO_4$ and $FeSO_4$ @0.5% each at 40 DAS increased Zn content in stover compared to control.

Table 3. Effect of seed, soil and foliar application of Zn on Zn content in grain and stover of maize

Treatments	**Zn content in grain (mg kg^{-1})**	**Zn content in stover (mg kg^{-1})**
Main plots		
Control	36.41	47.65
Seed inoculation (*Bacillus subtilis*)	37.27	52.28
Soil application of $ZnSO_4$ @16.25 kg ha^{-1}	42.26	54.89
Seed inoculation (*Bacillus subtilis*) + soil application of $ZnSO_4$ @16.25 kg ha^{-1}	42.59	56.43
CD (*P*= 0.05)	4.76	4.16
Sub plots		
Control	36.65	49.06
Foliar spray @0.5% $ZnSO_4$ at 45 DAS	40.55	53.71
Foliar spray @0.5% $ZnSO_4$ at 75 DAS	38.10	53.61
Foliar spray @0.5% $ZnSO_4$ at 45 + 75 DAS	43.23	54.87
CD (*P*= 0.05)	2.89	4.23

Interactions were found to be non-significant.

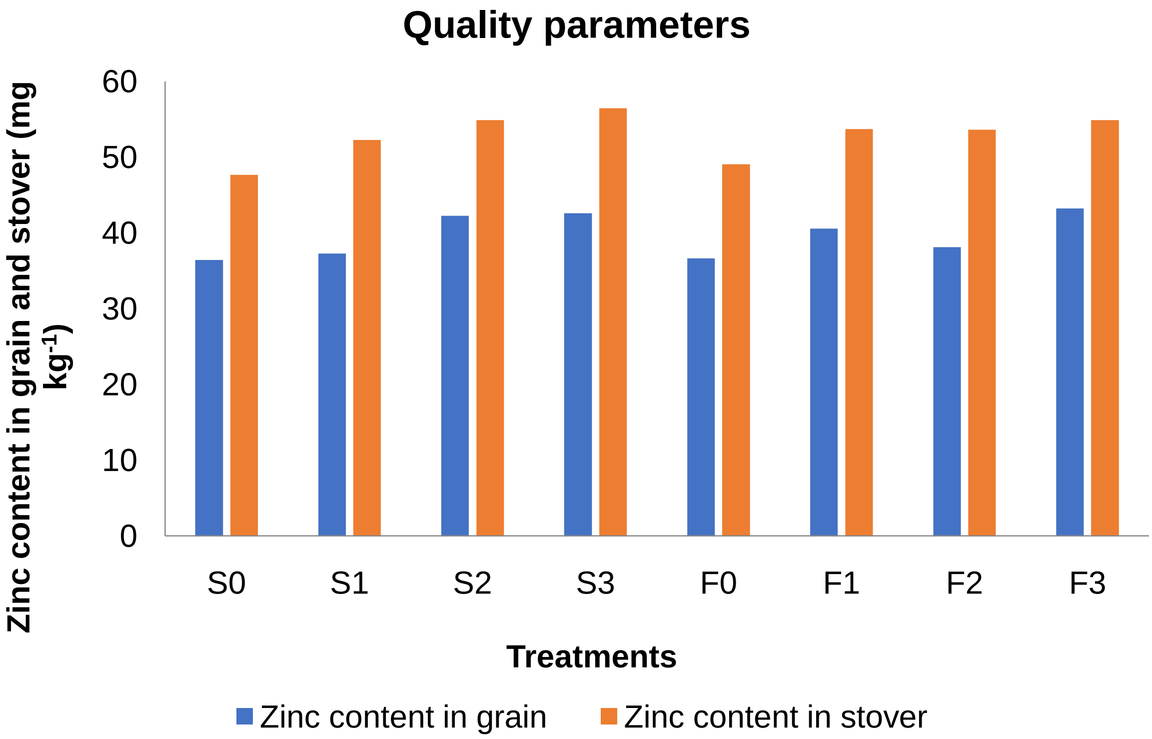

Fig. 2: Effect of seed, soil and foliar application of Zn on Zn content in grain and stover of maize

3.2.3 Zn uptake in grain (g ha^{-1})

Table 4 provide the findings about Zn uptake in grain. The results showed that treatment S_3 (seed inoculation (*Bacillus subtilis*) + soil application of $ZnSO_4$ @16.25 kg ha^{-1}) had the highest grain Zn uptake (245.4 g ha^{-1}) followed by treatment S_2 (soil application of $ZnSO_4$ @16.25 kg ha^{-1}), S_1 (seed inoculation (*Bacillus subtilis*)) and S_0 (control). The treatment S_3 showed significantly higher results than S_0 and S_1 but at par with S_2. Soil application of Zn @16.25 kg ha^{-1} also outperformed S_0 and S_1. Zn uptake in grain of S_3 increased by 4.87, 25.98, and 35.43 % compared to S_2, S_1 and S_0, respectively. According to Dwivedi *et al*. (2002), Zn has a crucial part to play in the production of enzymes and has a positive impact on plant metabolism, enabling plants to uptake more nutrients. Higher uptake of Zn is a result of improved mineralization of potassium and Zn by KSB and ZSB, which led to higher availability and high Zn content (Patil *et al*., 2022). The sub-plot treatment F_3 (foliar spray @0.5% $ZnSO_4$ at 45 + 75 DAS) had the highest grain Zn uptake (242.7 g ha^{-1}) while F_0 (control) had the lowest (186.3 g ha^{-1}). F_3 showed significantly better results than F_0, F_1 and F_2, meanwhile, F_1 showed significantly higher results than F_0 and F_2. Increases in Zn uptake in grain under F_3 was 7.87, 20.45, and 30.27 % over F_1, F_2, and F_0, respectively. The increased metabolic processes and enzyme activity caused by Zn nutrition play an important role in its uptake, which improves quality metrics. According to Arabhanviet *al*. (2018), foliar $ZnSO_4$ and $FeSO_4$ application @0.5% each at 40 DAS improved Zn uptake in grain when compared to control.

3.2.4 Zn uptake in stover (g ha^{-1})

Zn uptake by maize was significantly impacted by Zn treatment. The data is shown in table 4, which concluded that S_3 (seed inoculation (*Bacillus subtilis*) + soil application of $ZnSO_4$ @16.25 kg ha^{-1}) had significantly much higher Zn uptake in stover than S_0 and S_1. The treatment S_2 also gave better results than S_0 but showed very close results with S_1. Zn uptake in stover increased by 31.81, 16.20, and 5.63 % in the case of S_3 (seed inoculation (*Bacillus subtilis*) + soil application of $ZnSO_4$ @16.25 kg ha^{-1}) compared to S_0 (control), S_1 (seed inoculation (*Bacillus subtilis*) and S_2 (soil application of $ZnSO_4$ @16.25 kg ha^{-1}). In comparison to the control, Zn uptake

Table 4. Effect of seed, soil and foliar application of Zn on Zn uptake in grain and stover of maize

Treatments	**Zn uptake in grain (g ha^{-1})**	**Zn uptake in stover (g ha^{-1})**
Main plots		
Control	181.2	327.6
Seed inoculation (*Bacillus subtilis*)	194.8	371.6
Soil application of $ZnSO_4$ @16.25 kg ha^{-1}	234.0	408.8
Seed inoculation (*Bacillus subtilis*) + soil application of $ZnSO_4$ @16.25 kg ha^{-1}	245.4	431.8
CD (*P*= 0.05)	27.8	45.4
Sub plots		
Control	186.3	341.4
Foliar spray @0.5% $ZnSO_4$ at 45 DAS	225.0	398.3
Foliar spray @0.5% $ZnSO_4$ at 75 DAS	201.5	383.6
Foliar spray @0.5% $ZnSO_4$ at 45 + 75 DAS	242.7	416.4
CD (*P*= 0.05)	16.1	38.1

Interactions were found to be non-significant.

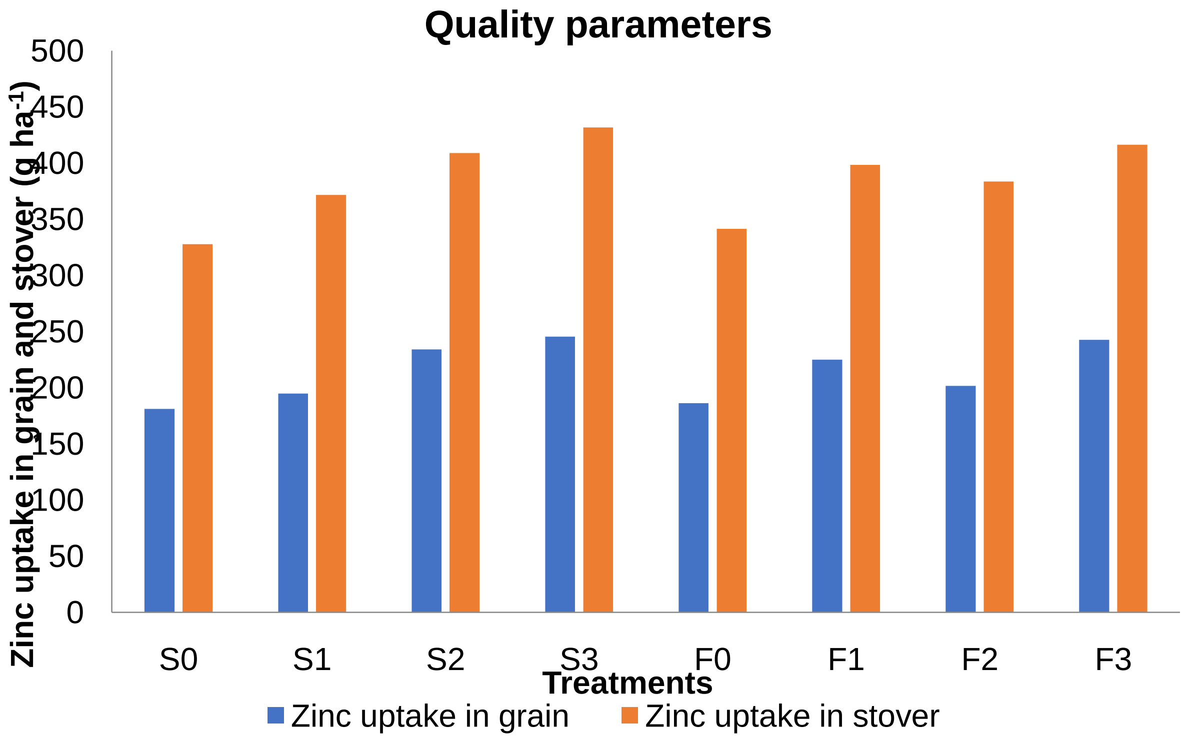

Fig. 3: Effect of seed, soil and foliar application of Zn on Zn uptake in grain and stover of maize

in fodder maize was increased when $ZnSO_4.H_2O$ @16 kg ha^{-1} was applied, according to Kumar *et al*. (2021). The fluctuation in applied Zn availability in the root zone and their function in the growth and development of the plant can both be attributed to the rise in Zn uptake that resulted from their increased application (Chaudhary and Sinha, 2007). Similar to this, treatment F_3 (foliar spray @0.5% $ZnSO_4$ at 45 + 75 DAS) recorded the highest stover Zn uptake (416.4 g ha^{-1}), whereas F_0 (control) recorded the lowest (341.4 g ha^{-1}). F_3 outperformed F_0 but gave closer results to F_1 and F_2. The foliar treatment F_1 was significantly higher than F_0 but gave the same results as F_2. The percentage increase in Zn uptake in stover for F_3 over F_1, F_2, and F_0 was 4.54, 8.55, and 21.96 %, respectively. According to Kumar *et al*. (2017), higher dry fodder yield and a significant rise in content both have the impact on enhancing Zn uptake. According to Kumar *et al*. (2017), Zn uptake in fodder maize was at its highest when one foliar spray of $ZnSO_4$ was provided at 30 DAS and two foliar applications were given at 30 & 45 DAS in comparison to control. According to Arabhanvi*et al*. (2018), foliar treatment of $ZnSO_4$ and $FeSO_4$ @0.5% each at 40 DAS improved Zn uptake in stover compared to control.

4. CONCLUSION

Based on the current research findings, it can be confidently concluded that the combination of seed inoculation with *Bacillus subtilis* along with the soil application of $ZnSO_4$ at a rate of 16.25 kg (S_3) has demonstrated a significant positive impact on both crop yield and quality parameters. Moreover, the application of $ZnSO_4$ through foliar at a content of 0.5% during two key stages of crop growth, specifically at 45 and 75 days after sowing (DAS) under treatment F_3, has also shown remarkable results in terms of enhancing both crop yield and quality parameters.

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