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Growth characteristics and Distribution of Zinc Fractions in Barley (*Hordeum vulgare* L.) Soil as Affected by Zinc-Based Fertilizer in Saline conditions

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ABSTRACT: The field experiment conducted during the rabi season of 2021-22 at the Agricultural Research Sub-station in Vallabhnagar, Udaipur, Rajasthan, aimed to assess the influence of zinc-based fertilizers on the growth characteristics and distribution of zinc fractions in the soil of barley (*Hordeum vulgare* L.) crop. The experimental design followed a split plot arrangement, with main plot treatments consisting of a control, 5 kg Zn per hectare as soil application, and seed treatment with zinc solubilizing bacteria (ZSB) at a rate of 5 ml per kg of seed. The sub plot treatments included a control and three foliar sprays of nano Zn at 5 ml per litre of water, applied at 15, 30, and 45 days after sowing. Each treatment was replicated three times. The soil application of zinc @ 5 kg Zn ha⁻¹ along with foliar spray of nano Zn @ 5 ml per litre of water sowing had significantly influenced plant height and dry matter accumulation at 60 DAS of barley as well as zinc fraction of soil after harvest of barley crop. the combination of conventional zinc fertilizer and foliar spray of nano zinc offers a promising strategy to improve nutrient availability and enhance zinc fraction distribution in the soil after the barley crop's harvest. This approach holds potential for optimizing zinc uptake by barley plants and promoting sustainable agricultural practices in regions with zinc-deficient soils.

Keywords: Foliar application, Soil Application, Nano Zinc, Barley, Zinc Fraction.

INTRODUCTION

Barley (*Hordeum vulgare* L.) is a significant cereal crop in India, renowned for its adaptability to diverse agro-climatic conditions. It has been cultivated in the country for centuries and holds an important place in Indian agriculture. Barley serves multiple purposes in India's agricultural landscape. It is grown as a food crop, animal feed, and also for malt production in the brewing industry. The crop is known for its resilience to drought conditions and its ability to grow well in relatively cool and dry regions, making it a suitable option for farmers facing water scarcity or those seeking crop diversification (Rizwan *et al.*, 2020).

In recent years, the cultivation of barley in India has witnessed fluctuations due to various factors. The total area under barley cultivation has shown some variability, with fluctuations based on market demand, weather patterns, and the availability of irrigation facilities. However, barley cultivation remains prominent in states such as Rajasthan, Uttar Pradesh, Madhya Pradesh, Haryana, and Punjab. Barley occupies 0.46% of the total cropped area, 0.62% of the food grains and 0.76% of the cereals in the country. Similarly, it contributes 0.86 of the total production of cereals and 0.81% of the food grains in India. Area under coarse cereal crops has reduced from 37.67 million hectares in 1950-51 to 24.15 million hectares in 2019-20. However, production has increased from 15.38 million tonnes to 41.75 million tonnes during the same period (GoI, 2021). Yield of barley increased from 1938 kg ha⁻¹ in 2005-06 to 2881 kg ha⁻¹ in 2019-20 (DAC and FW 2020).

Micronutrients form the foundation of plant nutrition in developed countries, playing a pivotal role in agriculture. The significance of proper plant nutrition cannot be overstated as it stands as one of the foremost factors in enhancing both the quality and quantity of crop yields. Despite the judicious use of NPK fertilizers, achieving optimal crop yields remains a challenge due to the deficiency of micronutrients, particularly zinc (Zn). Zinc is an essential micronutrient for plants, but Indian soils generally exhibit low availability of Zn, making it a significant constraint to crop productivity. Over the past four decades, Zn deficiency in India has increased from 42-49%, and it is projected to rise to 63% by 2025 (Cakmak *et al.*, 2020). This deficiency is not limited to India alone, as onethird of the world's population is at risk of Zn malnutrition due to inadequate dietary intake of Zn (Hafeez et al., 2021). Zinc in the soil exists in various chemical forms, each differing in solubility and thus impacting its availability to plants. In order to establish a comprehensive understanding of zinc (Zn) dynamics in soil, it is essential to consider the different fractions of zinc present. Among these fractions, the watersoluble, exchangeable, and adsorbed pools are believed to exist in a state of reverse equilibrium, with rapid establishment of equilibrium between them. The availability of applied zinc to plants is significantly influenced by the transformations that occur within the soil, including the quantity and rate of these transformations. Factors such as the amount of nutrients added, reaction time in the soil, rate of extraction by plant roots, nature and quantity of clay minerals, and organic matter content all play a crucial role in determining the extent and nature of these transformations.

Plants uptake zinc from the soil solution, which is continuously replenished by various zinc fractions. Therefore, it is imperative to assess the different forms of zinc, their relationships with one another, and their uptake by plants for sustainable crop productivity over the long term. With this in mind, the present investigation was conducted to study the impact of zinc fertilization on zinc fractions in saline soil at the Agricultural Research Sub-Station (ARSS), Vallabhnagar, Udaipur, Rajasthan. This research aimed to provide insights into the changes occurring in zinc fractions following zinc fertilization in a saline soil environment. By understanding these transformations, it becomes possible to develop effective strategies to optimize zinc availability and uptake by plants, ultimately leading to enhanced crop productivity in such challenging soil conditions.

MATERIALSAND METHODS

A. Location of experimental site

The experiment was conducted at the Agriculture Research Sub Station Farm, situated in Vallabhnagar, Udaipur, Rajasthan. The geographical coordinates of the location are approximately 24° 38' North latitude and 73° 42' East longitude. The experimental site is situated at an average altitude of 633 meters above sea level and is located just 45 km east of Udaipur, Rajasthan. The research site falls within agro-climatic zone IVa of Rajasthan, which is characterized as the Sub-Humid Southern Plain and Aravalli Hills region.

B. Experimental design and treatments

The experimental setup consisted of a split-plot design with three main plot treatments involving different zinc sources, and four sub-plot treatments involving the application of nano zinc at various time intervals. The experiment was replicated three times to ensure reliable results and reduce the impact of potential variability. This design allowed for the simultaneous evaluation of both the main plot treatments (zinc sources) and the sub-plot treatments (nano zinc application timing), enabling a comprehensive analysis of their individual and combined effects on the study variables.

C. Application protocol of fertilizers

In the experimental setup, the recommended doses of nitrogen (N) and phosphorus (P) were 60 kg ha⁻¹ and 20 kg ha⁻¹, respectively. These nutrients were applied using urea for nitrogen and diammonium phosphate (DAP) for phosphorus. Zinc was applied in the form of zinc sulphate, with the quantity varying based on the treatment. During sowing, the total amount of phosphorus and zinc, along with half of the nitrogen, were applied by placing them in furrows. The remaining half of the nitrogen was divided into two equal splits and applied during subsequent irrigations. Additionally, nano zinc was sprayed on the plants using a concentration of 5 ml per liter of water, following the specific treatment protocol. This foliar application of nano zinc was carried out as per the treatment requirements.

D. Growth Parameters

The periodical change in dry matter accumulation at 60 DAS was recorded by collecting whole plant samples from randomly selected observational rows of each plot in 1 m running length of row. These samples were dried in sunlight for 2-3 days and finally dried in an oven at 70°C till a constant weight was obtained. Thereafter, the samples were weighed for estimating total dry matter accumulation and their average was taken as dry matter accumulation and expressed in gram.

E. Zinc Fraction

To assess the distribution of zinc in different pools within the soil, a sequential fractionation procedure, as proposed by Iyengar and Deb (1977); Chandi and Takkar (1982), was employed. This procedure allows for the estimation of various zinc pools through a sequential extraction method.

Details of treatments and their symbols

	Symbols	
i.	Control	Zn ₀
ii.	5 kg Zn ha ⁻¹ (soil application)	Znsa
iii.	Zinc solubilizing bacteria @ 5 ml kg ⁻¹ of seed (Seed treatment)	Znst
i.	Control	NP ₀
ii.	Foliar spray of nano Zn @ 15 DAS	NP ₁₅
iii.	Foliar spray of nano Zn @ 30 DAS	NP30
iv.	Foliar spray of nano Zn @ 45 DAS	NP ₄₅

Sr. No.	Chemical pool studied	Extractant	Procedure details
1.	Water soluble plus exchangeable (Wsex-Zn) (mg kg ⁻¹)	1N NH4OAC (pH 7.0)	5 g of soil in a 50 ml centrifuged tube was shaken with 20 ml of the extractant for two hours and centrifuged 15 minutes at 2500 rpm.
2	Adsorbed zinc (Ads-Zn) (mg kg ⁻¹)	0.005 M DTPA (pH 7.3)	The residue of step A was shaken with 20 ml of DTPA extractant for four hours and centrifuged.
3.	Zinc associated with organic matter (Oc-Zn) (mg kg ⁻¹)	30% H ₂ O ₂ and 0.005 M DTPA (pH 7.3)	The residue of steps B in centrifuge tubes was allowed to react with 5 ml of 30 % H ₂ O ₂ and placed on a hot water bath overnight and the contents in the tube was maintained 75°C±2°C. After the completion of reaction, the soil was shaken with 20 ml of 0.005 M DTPA for two hours and centrifuged.
4.	Occluded zinc and Zn bounded by carbonates and other acid soluble minerals (Occ-Zn) (mg kg ⁻¹)	0.1 NHCl	The residue of step C was shaken with 20 ml 0.1 N HCl for one hour and centrifuged
5.	Residual zinc (Res-Zn) (mg kg ⁻¹)	-	Total zinc minus the sum of steps A B C and D
6.	Total zinc (T-Zn) (mg kg ⁻¹)	-	0.5 g of soil with a mixture of 10 ml of hydrofluoric acid, 3 to 6 drops of sulphuric acid and 5 ml of perchloric acid in Teflon beakers and analyzed by AAS (Page <i>et al.</i> , 1982).

Table 1: Sequential extraction of different Zn fraction.

F. Statistical Analysis

The experimental data were subjected to statistical analysis of variance using the procedure outlined by Panse and Sukhatme (1985). The 'F' test was employed to evaluate the significance of the observed differences. To compare the means, the critical difference (CD) was calculated at a 5% level of significance. This allowed for the interpretation and comparison of the findings in a statistically rigorous manner.

RESULT AND DISCUSSION

A. Growth parameters

The data presented in Table 2 revealed that soil application of zinc and seed treatment with zinc solubilizing bacteria had a significant impact on plant height at harvest of the barley crop over control. Soil application of zinc @ 5 kg Zn ha⁻¹ (Zn_{SA}) resulted in the maximum plant height (88.14 cm), while control (Zn_0) resulted in the minimum plant height (76.20 cm). The data also revealed that the plant height increased by 15.66 per cent due to soil application of zinc @ 5 kg Zn ha⁻¹ (Zn_{SA}), over control (Zn₀). Soil application of zinc and seed treatment with Z.S.B significantly affected crop dry matter accumulation at 60 DAS over control. Data clearly reflected that that soil application of zinc @ 5 kg Zn ha⁻¹ (Zn_{SA}) resulted in the maximum dry matter accumulation (59.49 g m⁻¹ row length) while control (Zn₀) resulted in the minimum dry matter accumulation (53.37 g m⁻¹ row length). The positive impact of applied zinc on these parameters can be attributed to its crucial physiological functions within the plants (Cakmak, 2008; Alloway, 2004). The promotion of growth through zinc application may be attributed to its involvement in various metabolic processes, facilitating catalytic activities in plants. Zinc plays a vital role in the synthesis of indole acetic acid, a precursor of auxin, which is responsible for enhancing plant height, branching, and overall dry matter accumulation in barley. Moreover, zinc is essential for

cellular growth, differentiation, and metabolism, which contribute to rapid plant growth, robust root development, and improved growth characteristics (Kuldeep *et al.*, 2018). Rana *et al.* (2019) also revealed that zinc fertilizer through soil application positively influences plant height and dry matter accumulation by promoting cell elongation, enhancing photosynthesis, and facilitating key metabolic processes. Ensuring an adequate supply of zinc to plants is crucial for maximizing their growth potential and optimizing biomass production, ultimately contributing to improved crop yields.

Foliar application of nano zinc had a significant effect on plant height of barley over the control. The maximum plant height (85.05 cm) was recorded with foliar spray of nano Zn at 45 DAS (NP₄₅) which was significantly higher than control (NP₀). The plant height of barley crop at harvest increased by 6.94 per cent due to application of foliar spray of nano zinc at 45 DAS (NP₄₅) over control (NP₀). Foliar application of nano zinc had a significant effect on dry matter accumulation of barley at 60 DAS over the control. The maximum dry matter accumulation (58.48 g m⁻¹ row length) was recorded with foliar spray of nano Zn at 45 DAS (NP₄₅) which was significantly higher than control (NP₀). The dry matter accumulation of barley crop at 60 DAS increased by 8.94 per cent due to application of foliar spray of nano zinc at 45 DAS (NP₄₅) over control (NP₀). The nanoscale size and high surface area-tovolume ratio of the zinc particles enhance their effectiveness in foliar application. This allows for better nutrient absorption, rapid translocation within the plant, and efficient utilization (Hasanuzzaman et al., 2020). Malik et al. (2020) found that foliar application of nano zinc fertilizer promotes increased plant height by stimulating cell elongation and division. The nanoparticles penetrate the leaf tissues and reach the cellular level, where they contribute to the synthesis of growth-promoting hormones such as auxins. These

hormones play a crucial role in cell elongation and expansion, resulting in taller plants. Nanoparticles with a size below 5 nm primarily follow the cuticular pathway, while nanoparticles with larger sizes tend to utilize the stomatal pathway for absorption by plant leaves (Eichert and Goldbach 2008). Subsequently, these nanoparticles are transported through the conducting system, facilitating the rapid and efficient absorption of nutrients by the leaves, as they are highly soluble (Fernández and Eichert 2009).

The combined application of soil and foliar nutrients has been observed to promote the improved growth of barley. This synergistic effect can be attributed to the supply of essential nutrients during the early stages through soil application, providing a strong foundation for emergence. Subsequently, the foliar application of nutrients allows for efficient nutrient utilization by the leaves, supporting enhanced vegetative and reproductive growth in barley (Amanullah *et al.*, 2014).

B. Soil zinc fraction

Data of Zn fractions analysis (Table 3) indicate that soil application of zinc and seed treatment with zinc solubilizing bacteria significantly increased the water soluble + exchangeable zinc (Wsex-Zn), adsorbed zinc (Ads-Zn), organically bounded zinc (Oc-Zn), occluded zinc (Occ-Zn), residual zinc (Res-Zn) and total zinc (T-Zn) content in soil over control after harvest of barley crop. The amount of the total Zn in the soil was increased significantly and it was ranged from 255.77 to 288.50 mg/kg. Another potential reason for the increased zinc (Zn) status of the soil could be attributed to the higher solubility, diffusion, and mobility of the soil applied inorganic Zn fertilizer. Inorganic Zn fertilizers typically have good solubility in water, allowing them to readily dissolve and become available for plant uptake.

The Residual Zn was the most dominant fraction, followed by organically bound Zn, occluded Zn, adsorbed Zn, while water soluble plus exchangeable Zn was the least dominant fraction. The control treatment exhibited the lowest residual Zn content, measuring 214.81 mg/kg. This can be attributed to the absence of Zn addition in the cropping system, resulting in the removal of Zn through uptake by the crop. Without the application of Zn fertilizer, the crop relies solely on the available soil Zn, leading to its depletion and lower residual Zn content in the soil. The higher solubility and mobility of the added ZnSO₄ may have contributed to the observed increase in the Ads-Zn fraction. This could be attributed to the dissolution of some precipitated Zn fractions upon Zn application. In general, the majority of the applied Zn entered the residual Zn pool, as the soil tends to adsorb and retain most of the applied Zn in a non-releasable form. Studies by Iyengar and Deb (1977) have shown that a significant portion of the fertilizer-derived Zn accumulates in the Occ-Zn and Res-Zn fractions.

Under saline conditions, the water-soluble and exchangeable Zn fractions are usually negligible. Similar findings were reported by Singh (2004); Jakhar (2006); Singh and Yadav (2007); Choudhary and Raina

(2008); Kumar and Basavaraj (2008); Preetha and Stalin (2014); Gajbhiye *et al.* (2018); Kumari *et al.* (2018); Farooq *et al.* (2021). The higher solubility, diffusion, and mobility of the applied inorganic zinc fertilizer could be a contributing factor to the increased Zn status of the soil. Similar results have been reported by Preetha and Stalin (2014); Tahir *et al.* (2019); Hafeez *et al.* (2020).

The foliar application of nano zinc had a significant positive impact on various zinc fractions in the soil after the harvest of the barley crop. Compared to the control, the foliar spray of nano Zn at 45 DAS (NP45) resulted in a notable increase in the water-soluble + exchangeable zinc (Wsex-Zn), adsorbed zinc (Ads-Zn), organically bounded zinc (Oc-Zn), occluded zinc (Occ-Zn), residual zinc (Res-Zn), and total zinc (T-Zn) content in the soil (Table 2). This improvement in the zinc fractions can be attributed to the high biodegradability of nano particles. The biodegradability of the nano particles leads to a higher rate of mineralization of both native and applied zinc, thereby increasing the concentration of zinc in the soil's zinc pool. Additionally, the foliar application of nano zinc introduces additional zinc content to the soil, further contributing to the enhanced zinc fractions.

The application of nano zinc fertilizer has been found to significantly influence the zinc fractions in the soil. Various zinc fractions, including water-soluble + exchangeable zinc (Wsex-Zn), adsorbed zinc (Ads-Zn), organically bounded zinc (Oc-Zn), occluded zinc (Occ-Zn), residual zinc (Res-Zn), and total zinc (T-Zn), have shown significant changes as a result of nano zinc fertilizer application (Zafar *et al.*, 2021).

Zia *et al.* (2019) have reported that the use of nano zinc fertilizer leads to an increase in the water-soluble + exchangeable zinc fraction, indicating improved availability of zinc in the soil solution. The adsorbed zinc fraction also shows an increase, suggesting enhanced binding of zinc to the soil particles. Moreover, the application of nano zinc fertilizer has been shown to increase the concentration of organically bounded zinc, occluded zinc, and residual zinc fractions in the soil, indicating their accumulation and retention in the soil matrix (Ullah *et al.*, 2018).

The impact of nano zinc fertilizer on the zinc fractions of soil is attributed to several factors, including the small particle size and high reactivity of nano zinc, which facilitates its dissolution and subsequent interaction with soil components. The biodegradability of nano particles also contributes to the release of zinc, enhancing its availability for plant uptake and influencing the distribution of zinc fractions in the soil (Farooq *et al.*, 2018).

The soil applied zinc fertilizer provides a readily available source of zinc, while the nano zinc fertilizer, with its small particle size and high reactivity, can further enhance zinc dissolution and interaction with soil components. This combined effect can lead to increased binding of zinc to soil particles, accumulation in organic matter, and retention in the soil matrix.

Table 2: Effect of different zinc sources on plant height and dry matter accumulation of barley.

Treatments	Plant height (cm)	Dry matter accumulation (g m ⁻¹ row length) At 60 DAS		
	At harvest			
Main Plot (Soil application and seed treatment)				
$Zn_0 = Control$	76.20	53.37		
$Zn_{SA} = 5 \text{ kg } Zn \text{ ha}^{-1}$ (soil application)	88.14	59.49		
$Zn_{ST} = Z.S.B. @ 5 ml kg^{-1} of seed (Seed treatment)$	85.22	57.65		
S Em±	1.48	1.18		
CD (P= 0.05)	5.80	4.63		
Sub Plot (Foliar application)				
$NP_0 = Control$	79.53	53.68		
$NP_{15} = Foliar$ spray of nano Zn at 15 DAS	83.42	56.75		
$NP_{30} = Foliar$ spray of nano Zn at 30 DAS	84.73	58.45		
NP_{45} = Foliar spray of nano Zn at 45 DAS	85.05	58.48		
S Em±	1.51	0.67		
CD (P=0.05)	4.49	1.98		

Table 3: Effect of different sources of zinc on distribution of zinc fractions in soil.

Treatment	Water soluble + exchangeable zinc (mg kg ⁻¹)	Adsorbed zinc (mg kg ⁻¹)	Organically bounded zinc (mg kg ⁻¹)	Occluded zinc (mg kg ⁻¹)	Residual zinc (mg kg ⁻¹)	Total zinc (mg kg ⁻¹)				
Main Plot (Soil application and seed treatment)										
$Zn_0 = Control$	0.557	4.13	23.41	12.86	214.81	255.77				
$Zn_{SA} = 5kg Zn ha^{-1}$ (soil application)	0.645	4.41	24.45	13.60	255.77	298.88				
$Zn_{ST} = Z.S.B. @ 5 ml kg^{-1} of seed (Seed treatment)$	0.610	4.27	23.95	13.26	240.41	282.50				
SEm±	0.005	0.024	0.113	0.126	1.779	1.842				
CD (P=0.05)	0.021	0.093	0.443	0.493	6.987	7.233				
Sub Plot (Foliar application)										
$NP_0 = Control$	0.591	4.15	23.03	12.91	219.33	261.00				
$NP_{15} = Foliar$ spray of nano Zn at 15 DAS	0.601	4.22	23.89	13.17	232.96	274.85				
NP_{30} = Foliar spray of nano Zn at 30 DAS	0.607	4.31	23.95	13.27	241.20	284.33				
$NP_{45} = Foliar spray of nano Zn at 45 DAS$	0.622	4.32	24.15	13.40	246.51	289.00				
SEm±	0.005	0.024	0.146	0.115	2.069	2.202				
CD (P= 0.05)	0.015	0.072	0.25	0.21	6.146	6.543				

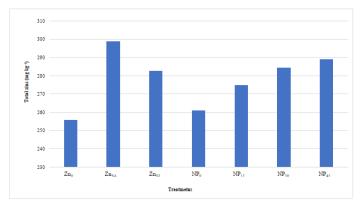


Fig. 1. Effect of different sources of zinc on total zinc fraction in soil at harvest of barley.

CONCLUSIONS

From the forgoing result, it was concluded that the combined application of the conventional and nano fertilizers significantly alters the growth parameter of barley and distribution of zinc fractions in post-harvest soil of barley crop. The combined application of conventional zinc fertilizer and nano zinc fertilizer can potentially enhance the distribution and availability of zinc fractions in the soil, providing an improved nutrient supply for plants and supporting their growth and development. The synergistic effect of both fertilizers can result in improved solubility, mobility, and bioavailability of zinc in the soil, leading to enhanced zinc fractionation. The combined application

of conventional zinc fertilizer and nano zinc fertilizer demonstrated promising results in altering growth parameters of barley and influencing the distribution of zinc fractions in post-harvest soil. This approach has the potential to enhance nutrient availability, promote plant growth, and optimize nutrient management practices in agricultural systems.

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