Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/7zpwtx53

AGRONOMIC APPROACHES FOR ZINC BIOFORTIFICATION IN RICE: EVALUATING SOIL, SEED, AND FOLIAR METHODS

Muhammad Saleem[1*], Iqtidar Hussain[1], Muhammad Aslam[2]

[1]Department of Agronomy, Faculty of Agriculture Gomal University, D. I. Khan, Pakistan
[2]Fodder Research Institute, Sargodha, Pakistan

***Corresponding Author:** Muhammad Saleem
*Email: salluji125@gmail.com

Abstract
Zinc (Zn) is a vital trace element that plays a crucial role in human health, particularly in enhancing immune function and protecting against infectious diseases. To combat zinc malnutrition, several strategies have been adopted globally, including dietary diversification, food fortification, supplementation, and biofortification. Among these, biofortification of crops, particularly rice, with zinc has emerged as the most effective and sustainable solution. Rice, being a staple food for millions in South Asia, is a key target for biofortification due to its low inherent zinc content and widespread consumption. By enriching rice with zinc, biofortification addresses both malnutrition and soil fertility issues, aligning with global efforts to achieve food security and improve public health outcomes. In this context, a field study was conducted to evaluate the effectiveness of various zinc application methods in rice cultivation. Zinc was applied through soil application, seed priming, and foliar spraying, with the aim of enhancing zinc content in rice grains while improving growth and yield. The study demonstrated that zinc (Zn) fortification through soil application, seed priming, and foliar spraying significantly enhanced rice growth, yield, and grain zinc concentration. Soil application of $ZnSO_4$ at 15 kg ha^{-1} proved highly effective in improving key growth parameters, such as leaf area index (LAI), leaf area duration (LAD), crop growth rate (CGR), and net assimilation rate (NAR). It also enhanced agronomic traits, including increased plant height, a higher number of total tillers, and greater fertile tillers, resulting in substantial yield improvements, with paddy yields reaching 3.61 t ha^{-1} in 2015 and 3.95 t ha^{-1} in 2016. Seed priming with 1.5% $ZnSO_4$ promoted early heading and maturity, improved early growth, and strengthened seedling vigor. Foliar spraying of 1.5% $ZnSO_4$ efficiently corrected zinc deficiencies during critical growth stages by supplying zinc directly to the leaves, which improved leaf health and photosynthesis, further supporting higher yields. Additionally, zinc fortification across all methods significantly increased zinc concentration in rice grains while reducing panicle sterility and improving grain quality. These results underscore the effectiveness of zinc fortification in enhancing rice growth, yield, and nutritional value, making it a sustainable strategy for addressing zinc deficiency in rice-based cropping systems.

Introduction
Zinc (Zn) is a vital micronutrient that plays a fundamental role in the growth and development of plants, including rice (*Oryza sativa* L.), which serves as a staple food for more than half of the global population. Zinc deficiency not only hampers plant growth and yield but also compromises the nutritional quality of grains, thereby affecting human health. Zinc deficiency in humans is a global concern, particularly in regions where rice is a primary dietary staple. Insufficient dietary zinc intake

leads to weakened immunity, stunted growth, and increased vulnerability to infections. Addressing this dual challenge of zinc deficiency in plants and humans requires innovative and sustainable agricultural interventions.

Rice (*Oryza sativa* L.) is a staple food crop that sustains a significant proportion of the world's population, particularly in Asia (Cordero-Lara, 2020; Ghanghas et al. 2022). Ensuring high productivity and quality of rice is essential to meet the growing food demands and to support global food security (Mohidem et al. 2022; Rezvi et al. 2023). Rice is predominantly grown in flooded conditions, which alter the chemical properties of the soil, rendering zinc less available due to the precipitation of insoluble zinc compounds. Moreover, factors such as high soil pH, low organic matter, and excessive reliance on chemical fertilizers exacerbate the problem. As a result, zinc deficiency is widespread in rice-growing regions, leading to yield losses and reduced grain quality.

Agronomic biofortification has emerged as a practical solution to combat zinc deficiency in rice. It involves applying zinc through soil, seed treatments, or foliar sprays to improve zinc uptake by plants and enhance its concentration in edible parts. Agronomic biofortification leverages existing farming practices and resources, making it more accessible and cost-effective for smallholder farmers. This approach not only improves crop productivity but also contributes to addressing micronutrient deficiencies in human diets. Common methods include soil application, foliar sprays, and seed priming, each with its advantages and limitations. The comparative efficacy of these methods can vary depending on soil properties, environmental conditions, and rice varieties (Khampuang et al. 2021; Zhang et al. 2021). Research indicates that while both soil and foliar Zn applications can enhance rice performance and grain quality (Shrestha et al. 2020; Islam et al. 2024), their efficacy varies based on factors like rice variety and soil conditions. Despite the widespread adoption of these methods individually, there is limited research comparing their relative effectiveness in enhancing rice growth, yield, and grain zinc concentration. Understanding the comparative performance of these methods is critical for developing region-specific recommendations for farmers and policymakers.

This study aims to evaluate the impact of soil application, seed priming, and foliar application of zinc on rice growth, yield, and zinc biofortification. The objectives of this research are:

1. To determine the effectiveness of each method in enhancing plant zinc uptake and grain concentration.
2. To assess their influence on key agronomic parameters, including growth and yield.
3. To identify the most suitable approach for zinc biofortification in zinc-deficient rice-growing regions.

Materials and Methods

The experiment was carried out in a farmer’s field located at Tibba Hamid Shah, Tehsil Darya Khan, District Bhakkar, Punjab, Pakistan, during the 2015 and 2016 cropping seasons. Prior to the commencement of the experiment, soil samples were collected from multiple locations within the experimental area at depths of 0~15 cm and 16~30 cm using an auger. The physicochemical properties of experimental soil are summarized in Table 1. A randomized complete block design was employed. Treatments included zinc (zinc sulfate = 21%) application as soil application (15 kg ha^{-1}), foliar spray (1.5%) and seed priming (1.5%). For seed priming, rice seeds were soaked in liters of water with a 1.5% zinc sulfate solution keeping the ratio of seed and water as 1:3. The seeds were soaked for 10 hours, then removed and spread in the shade to dry to their original weight. After drying, the seeds were packed in bags and taken to the field for nursery planting. Each treatment was replicated four times, and the net plot size of each treatment was 1.4 × 5 meters. The rice variety used for the experiment was Basmati-515, a fine rice variety.

Prior to seedling transplanting in field, the land was prepared through two rounds of cultivation, followed by planking. This was then complemented with irrigation and puddling to ensure optimal soil conditions. Thirty-day-old seedlings of the fine rice variety Basmati-515 were manually transplanted on June 25 in 2015 and 2016. The rice seedlings were planted by keeping a row spacing of 22.5 cm, with two seedlings per hill. Nitrogen (N) phosphorus (P) and potash (K) fertilizers were applied at rates of 160 kg ha^{-1} 100 kg ha^{-1} and 70 kg ha^{-1}, respectively. All P and K was applied as a basal dose while nitrogen (N) was applied in split doses: the first dose was applied 15 days after transplantation, the second dose at 30 days after transplantation, and the third dose at 45 days after transplantation. Weeds were managed manually throughout the growing season, and no herbicides were used.

Growth and phenological traits

Growth and phenological characteristics were observed during the study. The number of days taken to heading was noted from sowing until 50% of the crop had begun heading. Measurements were taken from three different sites within each plot, and the values were averaged. Similarly, number of days from heading to maturity was also noted from various sites of each treatment. In rice, tiller emergence rate significantly influences yield potential by determining the number of productive tillers, which directly contribute to grain formation. To determine the number of tiller emergence rate, five tillers from each treatment were tagged. Their emergence was recorded on a weekly basis until the completion of tillering. Leaf area index (LAI) is very important parameter that directly influences photosynthesis and determine the capacity of plants to intercept sunlight and convert it into biomass. The LAI was determined from 45 days after sowing to grain filling by measuring leaf area of three random samples per plot using a leaf area meter (CI-202, China). LAI was subsequently calculated following the method described by Hunt (1978).

Leaf area index (LAI) = $\frac{\text{Leaf area}}{\text{Ground area}}$

Similarly, leaf area duration (LAD) was calculated by averaging the leaf area indices (LAI) measured at two distinct time periods.

LAD = $[(LAI_1 + LAI_2) \times (t_2 - t_1)$ ½

Where LAI_1 = Leaf area index at first time, t_1 = Time of first LAI, LAI_2 = Leaf area index at final time, and t_2 = Time of final LAI.

Crop growth rate (CGR) quantifies the rate at which a plant increases its dry matter. It was determined by measuring the dry weight of plant samples collected at regular intervals from 1 m^2 plots every two weeks starting 45 days after planting. Random plant samples were collected from each plot and their fresh weight was immediately recorded. Subsequently, these samples were oven-dried at a constant temperature (e.g., 70°C) to a constant dry weight. Crop growth rate was then calculated using the formula:

Crop growth rate (g m^{-2} day^{-1}) = $\frac{W_2 - W_1}{t_2 - t_2}$

Where W_1 = plant dry weight at time t_1, W_2 = plant dry weight at time t_2. t_1 = Time of 1st harvest and t_2 = Time of 2nd harvest.

Net assimilation rate (NAR) quantifies the rate at which a plant accumulates dry matter per unit leaf area over time. The NAR was determined by following formula (Hunt, 1978).

Net assimilation rate (g m^{-2} day^{-1}) = TDM / LAD

Where TDM shows total dry matter while LAD represents leaf area duration.

Plant nutrient analysis

For zinc analysis, plant leaves from each treatment were randomly collected and cleaned with ultra-pure water upon arrival at the laboratory. The samples were then prepared by digestion following the method outlined by Jones and Case (1990). The digested samples were analyzed using an atomic absorption spectrophotometer (model 280FS AA) in accordance with the standard procedure described by Norhaizan and Ain (2009). To ensure accurate measurement, working standards of zinc were prepared at concentrations of 0.5, 1.0, 1.5, 2.0, and 2.5 mg L^{-1}. These standards were made from a stock solution of 1000 mg L^{-1} Zn, which was prepared using $ZnSO_4 \cdot 7H_2O$ salt diluted with distilled water. The standards were used to calibrate the atomic absorption spectrophotometer by running them to generate a calibration curve. Once the calibration curve was established, the prepared plant samples were analyzed. The zinc concentration in the plant samples was determined by running them on the spectrophotometer and recording the readings. The final zinc concentration in the plant samples was calculated by multiplying the observed sample readings by the dilution factor.

Agronomic and yield related traits

Agronomic and yield traits are crucial for optimizing crop productivity and profitable agriculture. Plant height was assessed by measuring the vertical distance from the base of the plant to the tip of the flag leaf. This was done for five main productive tillers selected at random from three locations within each plot to ensure representativeness. Similarly, number of productive tiller counts were performed within 1 m^2 quadrants at three separate sites per plot to obtain accurate data. For number of branches and grains (unhulled grain) per panicle, 20 panicles from each treatment were randomly selected, and the number of branches and grains were meticulously recorded to evaluate panicle structure and productivity. Likewise, the weight of 1000 grains were determined by averaging measurements from three samples per treatment, providing a reliable estimate of 1000 grains weight. Total grain and straw yields were calculated based on the harvested material from each plot, with results expressed in tons per hectare. Yield data were adjusted for moisture content to ensure accuracy and comparability.

Panicle Fertility and quality traits

To evaluate panicle fertility and quality traits, several measurements and analyses were conducted. Panicle sterility was assessed by comparing the number of viable paddies to the total number of paddies in a sample. Sterility percentage was calculated using the formula (Subedi et al. 1997).

Panicle sterility (%) = (a- b) / a × 100

Where “a” is the number of viable kernels and “b” is the total kernel count of panicle.

Normal kernels and chalky kernels were identified by their translucency and typical starch compaction. Kernels that passed light through and showed the characteristics were classified as normal. Both normal and chalky kernels were counted from random samples of the bulk yield collected from each replication plot. Kernel protein content was measured using the Micro-Kjeldahl method, which involved the digestion of the kernels, followed by ammonia distillation. The nitrogen concentration was determined through ten chlorimetric ammonia assays of the digest. The protein content was calculated by multiplying the nitrogen concentration by a factor of 5.95. Kernel dimensions, including length and width, were measured using a digital caliper.

Results

The application of zinc (Zn) significantly influenced rice growth and development. Zn seed priming led to earlier heading and maturity compared to foliar spray and soil-applied Zn at 15 kg ha^{-1}. Across both years, seed priming consistently resulted in the shortest durations for heading and maturity, demonstrating its effectiveness in accelerating crop development. Regarding growth parameters, soil-applied Zn at 15 kg ha^{-1} achieved the highest leaf area index (LAI), with values of 9.0 in 2015 and 9.4 in 2016. While foliar Zn and Zn seed priming also improved LAI, their effects were slightly lower than soil-applied Zn. Similarly, soil-applied Zn extended the leaf area duration (LAD), reaching 293 days in 2015 and 292 days in 2016, outperforming foliar Zn and Zn seed priming, which resulted in shorter durations. These findings highlight the critical role of zinc application methods in enhancing rice growth and development, with soil-applied Zn delivering the most pronounced benefits in LAI and LAD, while Zn seed priming showed promise for improving early growth and crop maturity.

The results of zinc (Zn) application methods revealed significant improvements in rice growth, yield, and quality. Soil-applied Zn at 15 kg ha^{-1} consistently demonstrated superior performance across various parameters. It resulted in the highest crop growth rate (CGR) values of 29.4 and 29.9 g m^{-2} day^{-1} in 2015 and 2016, respectively, and also achieved the highest net assimilation rate (NAR) values of 7.7 and 7.6 g m^{-2} day^{-1} during the same years. Additionally, soil-applied Zn significantly enhanced plant height, measuring 118.5 cm in 2015 and 120.4 cm in 2016, and produced the greatest total tiller counts of 625 and 630, along with the highest number of fertile tillers, 525 and 529, in 2015 and 2016, respectively.

Zn seed priming and foliar application also improved growth and yield parameters but were comparatively less effective. Zn seed priming promoted earlier heading and maturity, while soil-applied Zn led to the highest yield improvements. Paddy yields reached 3.40 t ha^{-1} in 2015 and 3.60 t ha^{-1} in 2016 with soil-applied Zn, outperforming other methods. In terms of grain quality, the basal application of 15 kg Zn ha^{-1} resulted in the lowest panicle sterility rates and the highest protein content, achieving 7.79% in 2015 and 7.77% in 2016. This method also significantly increased zinc concentration in rice leaves and grains, with leaf Zn levels reaching 37.42 mg kg^{-1} and 37.46 mg kg^{-1} in 2015 and 2016, respectively. Foliar Zn application resulted in moderate improvements, while Zn seed priming showed the lowest impact on leaf Zn concentrations.

Overall, soil-applied Zn proved to be the most effective method for enhancing growth, yield, and zinc nutritional quality in rice, followed by foliar spray and seed priming. These findings highlight the critical role of zinc fortification in improving rice productivity and nutritional outcomes.

Discussion

The findings of this study highlight the critical role of zinc (Zn) application in enhancing the growth, yield, and quality of fine rice cultivars. Zinc, an essential micronutrient, is involved in various metabolic processes, including enzyme activation, protein synthesis, and photosynthesis. Adequate zinc availability supports improved plant health, productivity, and grain quality, as documented in previous studies (Sharma et al., 1990; Broadley et al., 2007). The basal application of $ZnSO_4$ at 15 kg ha^{-1} emerged as the most effective method for improving key agronomic traits such as plant height, tiller numbers, and yield components. It enhanced critical growth parameters, including leaf area index (LAI), crop growth rate (CGR), and net assimilation rate (NAR), resulting in higher biomass accumulation and improved overall plant performance. Soil-applied Zn supports extensive root development, facilitating better nutrient and water uptake, which in turn promotes increased photosynthetic activity (Ghoneim, 2016; Cheema et al., 2006).

Additionally, soil-applied Zn significantly improved grain quality traits, including higher protein content and reduced panicle sterility. These improvements are consistent with findings by Sharma et al. (1990) and Broadley et al. (2007), who reported enhanced grain quality and nutritional value with adequate zinc fertilization. The steady supply of Zn throughout the crop's growth cycle makes soil application particularly effective for addressing both agronomic and nutritional challenges.

Foliar application of $ZnSO_4$ proved beneficial for correcting zinc deficiencies during critical growth stages by delivering the nutrient directly to the leaves. This method bypasses soil-related challenges such as zinc fixation or unavailability. However, its impact on growth and yield was comparatively lower than that of soil application, as foliar-applied zinc tends to have a more immediate but short-lived effect. Despite its limitations, foliar spraying is valuable as a supplementary approach to address acute zinc deficiencies or provide additional nutrients during critical growth phases. Similar observations have been made in studies that emphasize the importance of timing and application technique for optimizing the benefits of foliar-applied zinc (Sadana & Takkar, 1985; Davis-Carter et al., 1991).

Seed priming with $ZnSO_4$ demonstrated its effectiveness in enhancing early seedling vigor and promoting faster germination. This method allows seeds to absorb zinc directly during the priming process, ensuring sufficient nutrient availability during the early stages of growth. The quicker heading and maturity observed with seed priming align with findings from Amanullah et al. (2009) and Azeem et al. (2018). However, its effects on later growth parameters, such as LAI, CGR, and NAR, were less pronounced compared to soil-applied zinc.

Seed priming is an economical intervention, particularly suitable for resource-constrained farmers, as it requires lower input quantities while ensuring improved seedling establishment.

Zinc application methods significantly influenced grain quality traits. The basal application of $ZnSO_4$ resulted in higher protein content and normal kernel percentages while reducing panicle sterility. These results are consistent with earlier studies that highlighted the role of zinc in improving grain nutritional quality and reducing sterility (Ghoneim, 2016; Sharma et al., 1990). Moreover, soil-applied Zn was associated with the highest zinc concentration in leaves and grains, demonstrating its efficacy in enhancing the crop's nutritional value. This reflects the potential of zinc fertilization to address zinc deficiencies in both soils and diets, contributing to human nutrition and public health, as emphasized by Broadley et al. (2007).

Conclusion

The results of this study underscore the critical role of zinc sulfate application in enhancing rice growth, yield, and grain quality. Soil-applied $ZnSO_4$ proved to be the most effective method for sustained nutrient supply and improved agronomic performance, while seed priming and foliar sprays provided targeted benefits at specific growth stages. These findings align with earlier research and provide valuable insights for developing sustainable rice cultivation practices that address zinc deficiency in agricultural systems and human diets.

Acknowledgements

I acknowledge my field and laboratory staff who helped me during my study.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Declaration of interest

The authors declare that they have no conflict of interest

Declaration of generative AI in scientific writing

During the preparation of this work the author(s) used Chat GPT in order to improve readability and language of manuscript. After using this tool/service, the author(s) reviewed and edited the content as needed and take(s) full responsibility for the content of the publication.

Author contributions

Conceptualization and Methodology by Iqtidar Hussain, Data curation, formal analysis and writing - original draft by Muhammad Saleem while review & editing by Muhammad Aslam.

References

1. Abdoli, M., Esfandiari, E., Mousavi, S.B., Sadeghzadeh, B., 2014. Effects of foliar application of zinc sulfate at different phenological stages on yield formation and grain zinc content of bread wheat (cv. Kohdasht). Azarian Journal of Agriculture 1, 11-17.
2. Adhikary, S., Biswas, B., Chakraborty, D., Timsina, J., Sarkar, S.K., 2022. Seed priming with selenium and zinc nanoparticles modifies germination, growth, and yield of direct-seeded rice (*Oryza sativa* L.). Scientific Reports 12, 12227. https://doi.org/10.1038/s41598-022-16475-x
3. Ahmed, W., Qaswar, M., Jing, H., Wenjun, D., 2020. Tillage practices improve rice yield and soil phosphorus fractions in two typical paddy soils. Journal of Soils and Sediments 20, 2906-2918.
4. Ali, H., Sarwar, N., Ahmad, S., Tariq, A.W., Shahzad, A.N., 2012. Response of wheat crop to phosphorus fertilizers and application methods grown under agro-climatic conditions of southern Punjab. Pak. J. Agri. Sci. 49, 485-489.
5. Alkahtani, J., Elshikh, M.S., Alwahibi, M.S., Muhammad, A., 2020. Phosphorus and zinc fertilization improve productivity and profitability of rice cultivars under rice-wheat system. Agronomy 10, 1942.
6. Alkahtani, J., Elshikh, M.S., Alwahibi, M.S., Muhammad, A., Alharbi, A., 2020. Phosphorus and zinc fertilization improve productivity and profitability of rice cultivars under rice-wheat system. Agronomy 10, 128. https://doi.org/10.3390/agronomy10010128
7. Alloway, B.J., 2013. Zinc in soils and crop nutrition. ISTE Ltd.

8. Amanullah, Asif, M., Sukhdev, S., Malhi, Riaz, A.K., 2009. Effects of phosphorus fertilizer source and plant density on growth and yield of maize in Northwestern Pakistan. Journal of Plant Nutrition 32, 2080-2093.
9. Amanullah, Inamullah, Alwahibi, M.S., Elshikh, M.S., Muhammad, A., 2020. Phosphorus and zinc fertilization improve zinc biofortification in grains and straw of coarse vs. fine rice genotypes. Agronomy 10, 112. https://doi.org/10.3390/agronomy10010112
10. Azeem, K., Khan, A., Naz, F., Ilyas, M., Azeem, I., Anwar, F., Ahmad, W., 2018. Agricultural Research Technology 13, 27-39.
11. Broadley, M.R., Brown, P., Cakmak, I., Rengel, Z., Zhao, F., 2012. Function of nutrients: Micronutrients. In: Marschner's Mineral Nutrition of Higher Plants, 3rd edition. P. Marschner (Ed.). Academic Press, London, UK, pp. 208-265.
12. Cakmak, I., 2008. Zinc deficiency in cultivated crops and human health. Journal of Plant Nutrition and Soil Science 171, 571-582.
13. Chaudhuri, S.K., Malodia, L., 2017. Biosynthesis of zinc oxide nanoparticles using leaf extract of Calotropis gigantea: Characterization and its evaluation on tree seedling growth in nursery stage. Appl. Nanosci. 7, 501-512. https://doi.org/10.1007/s13204-017-0586-7
14. Cheema, Ullah, N., Khan, N.U., 2006. Effect of Zn on panicle structure and yield of coarse rice. Pak. J. Agri. Res. 1994, 33-37.
15. Cordero-Lara, K.I., 2020. Temperate japonica rice (*Oryza sativa* L.) breeding: History, present and future challenges. Chilean Journal of Agricultural Research 80, 303-314.
16. Davis-Carter, J.G., Parker, M.B., Gaines, T.P., 1991. Interaction of soil zinc, calcium and pH with zinc toxicity in peanuts. Plant and Soil 145, 339-347.
17. Ding, W., He, P., Zhang, J., Liu, Y., Xu, X., Ullah, S., 2020. Optimizing rates and sources of nutrient input to mitigate nitrogen, phosphorus, and carbon losses from rice paddies. Journal of Cleaner Production 252, 119733.
18. Ghanghas, N., MT, M., Sharma, S., Prabhakar, P.K., 2022. Classification, composition, extraction, functional modification and application of rice (*Oryza sativa*) seed protein: A comprehensive review. Food Reviews International 38, 354-383.
19. Ghoneim, A.M., 2016. Effect of different methods of Zn application on rice growth, yield and nutrients dynamics in plant and soil. Journal of Agriculture and Ecology Research International 6, 1-9.
20. Hussain, S., Maqsood, M.A., Rengel, Z., Aziz, T., Abid, M., 2013. Estimated zinc bioavailability in milling fractions of biofortified wheat grains and in flours of different extraction rates. Int. J. Agric. Biol. 15, 921-926.
21. Hunt, R., 1979. Plant growth analysis: The rationale behind the use of the fitted mathematical function. Annals of Botany 43, 245-249.
22. Iqbal, M., Aslam, M., Ranja, A.M., Akhtar, J., 2000. Salinity tolerance of rice as affected by Zn application. Pakistan J. Biol. Sci. 3, 2055-2057.
23. Islam, M.S., Rashid, M.H., Kabiraj, M.S., Islam, M.N., 2024. Foliar supplementation of phosphorus and zinc enhanced the yield of Boro rice. Journal of Aridland 14, 1-10.
24. Jiang, B., Jianlin, S., Sun, M., Hu, Y., Jiang, W., 2021. Soil phosphorus availability and rice phosphorus uptake in paddy fields under various agronomic practices. Pedosphere 31, 593-603.
25. Jones Jr, J.B., Case, V.W., 1990. Sampling, handling, and analyzing plant tissue samples. Soil testing and plant analysis 3, 389-427.
26. Kakar, K., Xuan, T.D., Noori, Z., Aryan, S., Gulab, G., 2020. Effects of organic and inorganic fertilizer application on growth, yield, and grain quality of rice. Agriculture 10, 320.
27. Kandil, E.E., El-Banna, A.A.A., Tabl, D.M.M., 2022a. Zinc nutrition responses to agronomic and yield traits, kernel quality, and pollen viability in rice (*Oryza sativa* L.). Frontiers in Plant Science 13, 839373.

28. Kandil, E.E., El-Banna, A.A.A., Tabl, D.M.M., 2022b. Zinc nutrition responses to agronomic and yield traits, kernel quality, and pollen viability in rice (*Oryza sativa* L.). Frontiers in Plant Science 13, 854271.
29. Khampuang, K., Lordkaew, S., Dell, B., 2021. Foliar zinc application improved grain zinc accumulation and bioavailable zinc in unpolished and polished rice. Plant Production Science 24, 123-135.
30. Khan, M.U., Qasim, M., Khan, I., 2004. Effect of Zn fertilizer on rice grown in different soils of Dera Ismail Khan. Sarhad Journal of Agriculture 23, 1033-1040.
31. Lin, Q., Lu, J., Zhang, J., Shan, X., 2012. Effects of zinc fertilization on rice yield and grain quality on Zn-deficient paddy soils in southern China. Plant and Soil 356, 283-290.
32. Liu, H.E., Wang, Q.Y., Rengel, Z., Zhao, P., 2015. Zinc fertilization alters flour protein composition of winter wheat genotypes varying in gluten content. Plant Soil Environ. 61, 195-200.
33. Mahmoud Soltani, S., Hosseini Chaleshtori, M., Ebadi, A., 2023. Effect of seed priming with zinc on seed germination characteristics, and morphological characters, and mineral content of rice tissues of Hashemi rice cultivar. Iranian Journal of Soil Science 47, 187-199.
34. Maqsood, M.A., Rahmatullah, S.K., Aziz, T., Ashraf, M., 2009. Evaluation of Zn distribution among grain and straw of twelve indigenous wheat (Triticum aestivum L.) genotypes. Pak. J. Bot. 41, 225-231.
35. Marcar, N.E., Graham, R.D., 1986. Effect of seed manganese content on the growth of wheat (*Triticum aestivum*) under manganese deficiency. Plant Soil 96, 165-173.
36. Mohidem, I., Yusoff, W.M.W., Isa, M.M.M., Nor, M.M., 2020. Influence of foliar applications of zinc and copper on physiological and biochemical changes in rice under copper stress. Rice Science 27, 285-293.
37. Monreal, C., Monreal, M., 2002. Nanotechnology for delivery of bioactive agents for plant productivity: In: Nanotechnology and the Environment. American Chemical Society, Washington, DC, pp. 193-205.
38. Niazi, N.K., Manzoor, M., Bibi, I., Shahid, M., Naidu, R., Rinklebe, J., Ok, Y.S., Iqbal, M., 2017. Phosphorus nutrition in plants under arsenic stress: Implications for agriculture and food safety. Agronomy 7, 67.
39. Norton, G.J., Beebout, S., Acuin, M.C., Loeppert, R.H., Farrel, T., Price, A.H., 2010. Effect of organic and mineral soil amendments on zinc uptake by rice grown under flooded conditions. Soil Science and Plant Nutrition 56, 437-445.
40. Pal, D., Mandal, B., Bhattacharyya, P., Mandal, L.N., 2000. Influence of phosphorus on the availability of native and applied zinc in submerged soils. Plant and Soil 220, 229-236.
41. Pandey, N., Gupta, B., Pathak, G.C., 2013. Enhancement of growth and yield of rice under zinc deficiency by foliar application of zinc oxide nanoparticles. Plant and Soil 360, 15-24.
42. Prasad, T.N.V.K.V., Sudhakar, P., Sreenivasulu, Y., Latha, P., 2012. Effect of nanoscale zinc oxide particles on the germination, growth and yield of peanut. Journal of Plant Nutrition 35, 905-927.
43. Rashid, A., Ryan, J., 2004. Micronutrient constraints to crop production in soils with Mediterranean-type characteristics: A review. Journal of Plant Nutrition 27, 959-975.
44. Rehman, A., Maqsood, M.A., Akhtar, M.E., Siddique, M., 2006. Residual effect of soil applied zinc on growth, yield and zinc concentration in wheat. Pak. J. Agric. Sci. 43, 16-19.
45. Shivay, Y.S., Prasad, R., 2020. Zinc-coated urea improves productivity and zinc uptake by scented rice. Agronomy Journal 112, 1530-1540.
46. Stevens, G.A., Finucane, M.M., De-Regil, L.M., Paciorek, C.J., Flaxman, S.R., Branca, F., Peña-Rosas, J.P., Bhutta, Z.A., Ezzati, M., 2013. Global, regional, and national trends in haemoglobin concentration and prevalence of total and severe anaemia in children and pregnant and non-pregnant women for 1995-2011: a systematic analysis of population-representative data. Lancet Glob. Health 1, e16-e25.

47. Subba Rao, N.S., 1999. Soil microorganisms and plant growth. Oxford & IBH Publishing Co. Pvt. Ltd.
48. Tariq, A., Mott, C.J.B., Syers, J.K., 1993. The significance of mineral weathering as a source of phosphorus in sandy soils of central Queensland, Australia. Soil Science Society of America Journal 57, 1393-1397.
49. Thavarajah, D., Thavarajah, P., Sarker, A., Vandenberg, A., 2009. Lentils (Lens culinaris Medikus Subspecies culinaris): A whole food for increased iron and zinc intake. J. Agric. Food Chem. 57, 5413-5419.
50. Tomar, S.S., Singh, R.S., Sharma, H.P., 1991. Influence of phosphorus and zinc on lentil (Lens culinaris) yield, protein content, and nutrient uptake in soils of Madhya Pradesh. Indian Journal of Agricultural Sciences 61, 91-93.
51. Welch, R.M., Graham, R.D., 2002. Breeding crops for enhanced micronutrient content. Plant and Soil 245, 205-214.
52. Yamauchi, M., Win, T., Tin, H., Yoshida, S., 1990. Zinc deficiency in the rice plant. Japan Agricultural Research Quarterly 24, 8-14.
53. Zahoor, R., Ur Rehman, M.S., Awais, M., Rengel, Z., Malik, S.A., 2014. Zinc biofortification in rice: Leveraging agriculture to moderate hidden hunger in developing countries. Archives of Agronomy and Soil Science 60, 1573-1590.

Table 1. Physicochemical analysis of experimental soil of Tibba Hamid Shah

Characteristics	**Unit**	**Value**
Texture		Sandy loam
pH		8.2
EC	dS m^{-1}	0.28
Organic matter	%	0.85
Nitrogen (N)	%	0.045
Phosphorus (P)	ppm	8
Potassium (K)	ppm	109
Zinc (Zn)	ppm	0.4

Table 2: Effect of Zn application methods on number of days taken to heading, maturity and leaf area index

Parameters	2015			2016		
	Days taken to heading (days)	Days from heading to maturity (days)	LAI	Days taken to heading (days)	Days from heading to maturity (days)	LAI
$ZnSO_4$ @ 15 kg ha^{-1} mixed with soil	109.2 a	37.0 a	9.0 a	109.8 a	37.9 a	9.4 a
Seed priming with $ZnSO_4$ (1.5%)	102.3 d	35.2 c	8.4 b	104.2 c	36.1 c	8.6 b
Foliar spray of $ZnSO_4$ (1.5%) at tillering stage	105.3 b	36.0 b	8.3 b	106.1 b	36.6 b	8.5 b
LSD at 0.05 probability level	0.62	0.52	0.15	0.52	0.40	0.13

Table 3: Effect of Zn application methods on leaf area duration, crop growth rate (CGR) and net assimilation rate (NAR)

Parameters	2015			2016		
	LAD (days)	CGR (g m^{-2} day^{-1})	NAR (g m^{-2} day^{-1})	LAD (days)	CGR (g m^{-2} day^{-1})	NAR (g m^{-2} day^{-1})
$ZnSO_4$ @ 15 kg ha^{-1} mixed with soil	293 a	28.8 a	7.7 a	292 a	29.3 a	7.6 a
Seed priming with $ZnSO_4$ (1.5%)	288 b	27.6 b	7.3 b	288 b	27.8 b	7.3 b
Foliar spray of $ZnSO_4$ (1.5%) at tillering stage	285 c	27.1 c	7.0 c	286 c	27.3 c	6.9 c
LSD at 0.05 probability level	1.29	0.40	0.13	1.47	0.43	0.17

Table 4: Effect of Zn application methods on plant height, number of tillers and number of fertile tillers

Parameters	2015			2016		
	Plant height (cm)	Number of tillers (m^{-2})	Number of fertile tillers (m^{-2})	Plant height (cm)	Number of tillers (m^{-2})	Number of fertile tillers (m^{-2})
$ZnSO_4$ @ 15 kg ha^{-1} mixed with soil	118.5 a	625 a	525 a	120.4 a	630 a	529 a
Seed priming with $ZnSO_4$ (1.5%)	113.7 b	595 b	508 b	115.1 b	605 b	511 b
Foliar spray of $ZnSO_4$ (1.5%) at tillering stage	109.9 c	579 c	487 c	108.1 c	584 c	488 c
LSD at 0.05 probability level	1.33	12	6.90	1.21	10	7.60

Table 5: Effect of Zn application methods on branches per panicle, kernels per panicle and 1000-grain weight

Parameters	2015			2016		
	Branches per	Kernels per panicle	1000-grains weight (g)	Branches per	Kernels per panicle	1000-grains weight (g)
$ZnSO_4$ @ 15 kg ha^{-1} mixed with soil	12.5 a	80.4 a	16.5	12.6 a	79.8 a	16.4
Seed priming with $ZnSO_4$ (1.5%)	11.5 b	75.9 b	16.4	11.5 b	74.8 c	16.3
Foliar spray of $ZnSO_4$ (1.5%) at tillering stage	11.2 c	76.7 b	16.5	11.0 c	75.5 b	16.3
LSD at 0.05 probability level	0.25	0.80	Ns	0.20	0.86	Ns

Table 6: Effect of Zn application methods on panicle sterility, normal kernel and kernel yield

Parameters	2015			2016		
	Panicle sterility (%)	Normal kernel (%)	Kernel yield (t ha^{-1})	Panicle sterility (%)	Normal kernel (%)	Kernel yield (t ha^{-1})
$ZnSO_4$ @ 15 kg ha^{-1} mixed with soil	7.3 a	66.9 a	3.40 a	7.3	67.2 a	3.60 a
Seed priming with $ZnSO_4$ (1.5%)	7.1 b	65.8 b	3.20 b	7.4	66.0 b	3.20 b
Foliar spray of $ZnSO_4$ (1.5%) at tillering stage	7.3 a	66.9 a	2.90 c	7.4	67.1 a	3.00 c
LSD at 0.05 probability level	0.14	0.25	0.13	Ns	0.30	0.12

Table 7: Effect of Zn application methods on protein, carbohydrate and grain zinc content

Parameters	2015			2016		
	Protein content (%)	Carbohydrates (%)	Grains Zn concentration (mg/kg)	Protein content (%)	Carbohydrates (%)	Grains Zn concentration (mg/kg)
$ZnSO_4$ @ 15 kg ha^{-1} mixed with soil	7.79 a	79.20 a	37.42 a	7.77 a	79.10 a	37.46 a
Seed priming with $ZnSO_4$ (1.5%)	7.46 b	78.16 b	25.42 c	7.35 b	78.35 b	25.45 c
Foliar spray of $ZnSO_4$ (1.5%) at tillering stage	7.50 b	78.20 b	30.20 b	7.35 c	78.32 b	29.85 b
LSD at 0.05 probability level	0.14	0.45	0.47	0.20	0.49	0.43