

Review Article

Meta Analysis on Effect of Zinc Biofortification on Crops and Human Health

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Introduction

Zinc is a fundamental minor component important for more than 300 zinc metalloenzymes and are important for numerous chemical reactions that are occurring in the body. such as carbohydrate metabolism, protein and DNA synthesis, protein digestion, bone metabolism, and endogenous antioxidant systems [1]. The Recommended Dietary Allowance (RDA) for those aged 19 and over is 8 mg for women and 11 mg for males per day. An additional 11 mg and 12 mg are needed during nursing and pregnancy respectively. The UL for zinc for all males

Abstract

Introduction: Zinc is a fundamental minor component important for more than 300 zinc metalloenzymes and are important for numerous chemical reactions that are occurring in the body. In Pakistan staple food i.e wheat is biofortified with zinc and it show positive response in prevention of zinc deficiency and increases crop zinc concentration.

Objectives: In this systematic review the biofortification of different crops is reviewed and as well the effect of biofortified zinc crop on human health is discussed.

Methodology: A meta-analysis was used to conduct a systematic study of the effects of zinc biofortification on human performance and health. Various search engines were used to find a total of 27 recent studies from 2015.

Results: Their findings indicate that zinc concentration has a beneficial influence on yield and boosts yield. When compared to the control treatment, zinc-biofortified seed boosted wheat grain yield in all different field experiments. Zinc biofortification through agronomic biofortification can be utilized to improve the nutritional value of food crops as well as their resilience to water shortages. Biofortification is a simple and inexpensive way to boost the zinc content in grains. Aside from that, it enhances items' functionality and increases their resistance to environmental factors. In Pakistan's food crisis, a solution can be found in this method. Twenty six percent of children have zinc levels that are less than 60 g/dl, which is a significant health concern. Geographically speaking, there is a very strong correlation between soil zinc deficiency and human diseases in populations, pointing to a growing demand for zinc biofortification of crops.

Conclusion: The review found that zinc biofortification improves wheat grain yield and quality, reducing soil zinc deficiency. Agrobiologically enrichment, involving timely delivery of zinc-based fertilizers, is an affordable solution for addressing Pakistan's hunger issue.

Keywords: Food fortification; Zinc deficiency; Zinc biofortification; Agronomic biofortification.

and females aged 19 years and older is 40 mg per day [2]. Adverse effect of zinc deficiency on human health causes stunting growth, impaired immune function, increase chances of infection and complication during pregnancy and childbirth Hotz, C., & Brown, K. H. [3]. "Biofortification" or "biological fortification" refers to nutritionally enhanced food crops with increased bioavailability to the human population that are developed and grown using modern biotechnology techniques, conventional plant breeding, and agronomic practices [4]. In Pakistan staple

food i.e wheat is biofortified with zinc and it show positive response in prevention of zinc deficiency and increases crop zinc concentration [5]. Zinc is also helpful in building antiviral immunity against COVID-19 disease and any other future outbreak [6].

Half of the global population are facing iron and zinc deficiencies because their major part of diet consists of cereal crops i.e., wheat, rice and maize [7]. According to the National Nutrition Survey, Zinc deficiency in Pakistan under five-year children is 18.6%. Rural children have higher prevalence (19.5%) than that of urban children (17.1%). The prevalence of zinc deficiency in Pakistan is 22.1%. Its prevalence is higher in rural population than that of urban population [8]. In Pakistan zinc deficiency is more common i.e., about 70 percent of agricultural land is zinc deficient [9]. Different experiments show that KP soil is 37 % Zn, 14% Fe and 60% B deficient, and Punjab soil is 57% Zn, 21% Fe and 50% B deficient [10].

Zinc malnutrition is a global issue that is prevalent in people whose diet consists of cereals-based food. Zinc malnutrition is a global issue that is prevalent in people whose diet consists of cereals-based food. Through agronomic biofortification with zinc we can add zinc in edible parts of crops. It is a practical and cost-effective approach to eliminate the zinc malnutrition problem [11]. Zn and other micronutrient deficiencies are also said to have a significant negative impact on the gross national product in underdeveloped nations by lowering productivity and raising healthcare expenses [12].

In this research we collected all the data related to biofortification, its effect on crops and also its role in preventing deficiencies and its efficiency in absorbing nutrients in the human digestive system. I gathered all the data, combined it and added it to single research. So, it can get easy for a layman to understand it.

Objectives

The objective of this review is to determine the effect of soil foliar application of zinc effect on crops quality and quantity.

To investigate the effectiveness of zinc biofortification in preventing zinc malnutrition.

Literature Review

The study was designed to look into how wheat's yield qualities and nutritional value are impacted by Iron (Fe) and Zinc (Zn). For this objective, the Galaxy-2013 variety was utilized. Soil application and foliar treatment (two sprays of three levels 0.1%, 0.2%, and 0.3%) were adopted to evaluate the effect of Fe and Zn in district Bahawalnagar during 2018-19 and 2019-20. In this investigation, an RCBD with four replications was adopted. The results showed that the physiological parameters (Leaf area index, Leaf area duration, Crop growth rate, and Relative growth rate) for which data were collected after 50 days of sowing showed non-significant results, while the physiological parameters for which data were collected after 100 days of sowing showed significant results. The treatment that used two 0.3% Fe sprays produced the best yield (4.9 t ha⁻¹) and raised the amount of Fe in grains by 31% compared to the control. The Zn concentration in grains increased by 43.8% in the mean treatment, compared to the control, after receiving two sprays of 0.3% Zn. When these nutrients were applied to the soil, encouraging outcomes were also shown in comparison to the control, but foliar sprays had a more substantial impact on the number

of tillers, spikelets per spike, and grains. This study came to the conclusion that by using this strategy, we may not only boost wheat productivity but also address the major problem of hunger that exists today, especially in underdeveloped countries [13].

Asif et al., [14] conducted a study to know the impact of biofortification of Calcium (Ca), Zinc (Zn) and Iron (Fe) on yield and quality of forage sorghum under field conditions. The investigation was conducted using a complete block design (RCBD). Control (no spray) were used in the experiment. The treatments were T1: Ca at 3%, T2: Zn at 2%, T3: Fe at 1%, T4: Ca at 3% + Zn at 2%, T5: Ca at 3% + Fe at 1%, T6: Zn at 2% + Fe at 1%, and T7: Ca at 3% + Zn at 2% + Fe at 1%. The results showed that when nutrients (Ca at 3% + Zn at 2% + Fe at 1%) were applied together, the plants' height (30.0%), number of leaves (53.6%), stem diameter (48.6%), leaf area per plant (77.2%), fresh biomass (48.2%), dry matter yield (120.8%), dry matter contents (49.1%), crude proteins (78.6%), and ash contents (120.8%) were all increased in comparison to the control (no spray). However, the control treatment yielded the highest. However, the control treatment yielded the highest Acid-Detergent Fiber (ADF) and Neutral Detergent Fiber (NDF) concentrations. Following the application of Ca at 3% and Fe at 1%, the contents of Ca and Fe increased. The highest plant Zn concentrations were Ca at 3%, Zn at 2%, and Fe at 1%. Based on the findings, it is determined that applying Ca at 3%, Zn at 2%, and Fe at 1% together increased forage sorghum quality and yield.

Ramzan et al., [15] conducted a study to assess the effectiveness of foliar and soil-supplied Zinc (Zn) and Iron (Fe) on all aspects of wheat growth and yield. For this Randomized Complete Block Design (RCBD) were used. The result of it concluded that Protein contents, copper, zinc iron, magnesium, calcium, harvesting index and biological yield, economical yield, thousand grain weight, number of grains per spike, number of spikelets per spike, spike length, number of tillers, the height of plants has increased when Zinc Sulfate (ZnSO₄) and Iron Sulfate (FeSO₄) were applied through soil and foliar application and as alone or in combined form. The combined foliar spray of 0.5% ZnSO₄ and 1% FeSO₄ considerably increased the maximum growth or quality features of wheat when compared to other Zn and Fe concentrations applied. This research concluded that one of the main agricultural techniques for boosting grains' levels of micronutrients and reducing hunger is biofortification. Through this we can also improve wheat quality. In comparison to soil application, foliar treatment is more suitable for making nutrients available to plants for optimum growth. It is advised to apply Zn and Fe together (0.5% ZnSO₄ and 1% FeSO₄) by foliar spray to increase wheat crop productivity and grain quality.

Bana et al., [16] conducted a field study in the Himalayan foothills region to determine the effects of Zn fertilization on basmati rice biofortification and nutrient usage efficiency. According to the findings of this study, the highest grain (3.46 t/ha) and straw (7.93 t/ha) yield of basmati rice was obtained applying 4.0% Zn-coated urea (ZnCU) + 0.2% Zn Foliar Spray (FS) using ZnSO₄ • 7H₂O. ZnCU increased rice productivity by about 25.4% on average when compared to Commercial Urea. The highest concentrations of Zn (35.93 and 81.64 mg/kg) and N (1.19 and 0.45%) were likewise produced by the same Zn fertilization treatment in the rice grain and straw, respectively. Also, compared to soil application, ZnCU application at 4.0% (ZnSO₄ • 7H₂O) had the highest N Usage Efficiency (NUE). Zn fertilization had a major impact on the elongation ratio and protein

concentration of grain alone and the corresponding Zn fertilization treatment yielded the maximum quality values of 1.90 and 7.44%, respectively. According to the study's findings, ZnCU would be a crucial, low-cost, and practical technique for raising yield, NUE, and biofortification, as well as for reducing the problems caused by Zn deficiency in the human diet in many poor economies.

Subbaiah et al., [17] conducted a study to investigate the effect of different concentrations of ZnO-nanoparticulates (50, 100, 200, 400, 600, 800, 1000, 1500, and 2000 ppm) on maize crop overall growth and zinc translocation in comparison to bulk ZnSO₄ and control. The outcome of this research shows that a t 1500 ppm of ZnO-nanoparticulates, the highest germination rate (80%) and seedling vigor index (1923.20) were recorded. In comparison to the control, the yield increased by 42% and by 2000 ppm of ZnSO₄ by 15%. Following the application of 100 ppm (31.05 ppm) and 400 ppm (35.96 ppm) of ZnO-nanoparticulates, higher zinc accumulation (35.96 ppm) in grains was observed. The findings of this research showed that ZnO-nanoparticulates significantly affect maize grain development, yield, and zinc content, a crucial aspect for human health.

Ahmad et al., [18] conducted an experiment to reduce the yield loss that occurs in Punjab due to delayed sowing of wheat. For this purpose, different combinations of foliar-applied bio-regulator and micronutrients treatment were performed on wheat crops that is Zinc (Zn), Boron (B), Thiourea (TU), Zn + B (ZnB), Zn + TU (ZnTU), B + TU (BTU), Zn + B + TU (ZnBTU) treatment were applied at booting and grain filling stages in delayed sown wheat in 2017–18 and 2018–19. The control was also kept in experiment. Their result is compared to control. The findings demonstrated that, in 2017 and 2018, ZnBTU treatment significantly raised leaf area index by 25.06% and 23.21% and spike length by 15.11% and 19.65% compared to CK. Moreover, compared to CK, the ZnBTU treatment increased 1000-grain weight by 21.96% and 22.01% in 2017 and 2018, respectively. Similar to ZnBTU treatments, which were statistically comparable to ZnB and ZnTU treatments, increased Zn, B, and N concentrations in straw and grain. Overall, compared to control, ZnBTU treatment enhanced photosynthetic rate by 46.67%, transpiration rate by 26.03%, stomatal conductance by 76.25% and decreased internal CO₂ by 28.18%. Moreover, ZnBTU outperformed in terms of grain yield in 2017–18 (25.05%) and 2018–19 (28.49%) compared to control. This study concluded that, applying ZnBTU to the leaves of delayed-sown wheat throughout the grain filling and booting stages may be a potential way to boost grain output.

Kirtika et al., [19] conducted a field experiment to investigate the effect of Zn fertilizer on maize Zn content. Randomized full block designs were used in the experiment. Hybrid maize was selected. At the sowing stage three levels of zinc (10, 20 and 30 kg ha⁻¹) treatment were applied to maize. The findings demonstrated that when Zn was applied to the soil or to the leaves, maize grain output and Zn content both increased. The highest grain yield (7.76 t ha⁻¹) was produced by the plots that received 30 kg of zinc per hectare, which is similar to the 7.64 t ha⁻¹ grain production from the plots that received 20 kg of zinc per hectare. The increase over control in the case of Zn is 55% and 51.8%, respectively. The results demonstrated that Zn application boosted maize grain output and Zn content when applied as a soil or foliar application. The study came to the conclusion that using zinc fertilizer is an effective way to increase corn output, grain concentration, and eventually corn quality.

Jiang et al., [20] conducted a study to know the effect of zinc biofortification on zinc bio accessibility in wheat, further their nutrition and mineral components were also examined. The findings showed that following digestion, whole flour had a significantly higher Zn concentration (by 30.58%–30.86%) and soluble Zn content (by 28.57%–42.86%). This development mostly affects fine bran, break flour, and reduction flour. After biofortification, the amounts of micronutrients like iron, calcium, and vitamins (B1, B6, and B9) as well as macronutrients like ash, lipids, and proteins rose. The concentrations of the vitamins B2 and B5 also decreased. Although dietary fibers and starch are the main sources of carbohydrates, coarse bran showed a dropping trend in total dietary fiber while break and reduction flour showed an increasing trend in starch. The molar ratio of phytates decreased; however, zinc did not significantly increase zinc bio accessibility. These findings may help create wheat types that are higher in micronutrients and have better nutritional qualities. These results can be used to develop wheat varieties that are higher in micronutrients and have better nutritional characteristics.

Akram et al., [21] evaluated the research to assessed the contribution of zinc on raising rice yield, as well as zinc concentration and bioavailability. The result of it estimated that ten rice genotypes received a base dosage of Zn (10 kg Zn ha⁻¹) in the form of ZnSO₄.H₂O. By factors of 1.08, 2.48, and 2.47, respectively, Zn application enhanced average grain production, grain Zn concentration, and absorption. Genotypes that were evaluated had an average Phytate to Zn ratio that had dramatically dropped by 57%. Additionally, the estimated human bioavailability of zinc from rice was raised. The highest Zn bioavailability yet observed was 4.27 mg/300g/day. Genotypes' average Zn bioavailability increased by a factor of 2.06 on average. In verdict, increasing crop production, Zn content, and bioavailability through agronomic Zn biofortification of rice may be a simple, flexible, and quick strategy.

In order to determine the effects of biofortification on the yield, quality, and Zn concentration of basil cultivars 'Aroma 2' and 'Eleonora' grown in a floating raft system, Ciriello et al., [22] conducted a study by using nutrient solutions with various Zn concentrations (12.5, 25.0, 37.5, and 50 M). By using UV-VIS spectrophotometry, researchers were able to measure the antioxidant activities of ABTS (2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate), DPPH (2,2-diphenyl-1-picrylhydrazyl), and FRAP (ferric reduction antioxidant potency), the tissue Zn concentration by inductively coupled plasma mass spectrometry and the concentrations of phenolic acids by mass spectrometry using a Q Exactive Orbitrap LC-MS/MS. The result of the study demonstrated that increasing the concentration of Zn in the nutrient solution significantly reduced the yield, this reduction was less evident in 'Aroma 2'. The study's findings showed that the yield was greatly decreased when the concentration of Zn in the nutrient solution was raised; this loss was less pronounced in "Aroma 2." But irrespective of cultivar, the application of the maximum Zn dose (50 M) raised the concentration of carotenoids, polyphenols, and antioxidant activity on average by 19.76, 14.57, and 33.72%, respectively, in comparison to the Control. The study concluded that basil is a good candidate for soilless biofortification programmes, as evidenced by the strong positive association between Zn in the nutrient solution and Zn in plant tissues.

In the Loess Plateau, a typical Zn, Se, and I deficient region of China, Mao et al., [23] conducted a study to examine the ef-

fects of soil and foliar applications of Se, Zn, and I on yield and concentrations of Se, Zn, and I in edible parts of various food crops including wheat, maize, potato, cabbage, and soybean. In one section of the trials, Se and Zn fertilizers were sprayed as a solution on winter wheat, while fertilizers of Se, Zn, and I were applied to soil either separately or together. The result of the study demonstrated that all of the crops that were evaluated responded well to the addition of selenium to the soil in the form of sodium selenate, whether alone or in combination with Zn and/or I. When applied topically to winter wheat, selenium as sodium selenite increased selenium content from 25 to 312 g kg⁻¹ in wheat grain with 60 g Se ha⁻¹. When it comes to Zn, soil-applied zinc sulfate was only shown to be beneficial in biofortifying cabbage leaves and canola seeds, boosting their Zn concentrations to 35 and 61 mg kg⁻¹, respectively. However, foliar zinc sulphate application was successful in biofortifying winter wheat, increasing the grain's Zn content from 20 to 30 mg kg⁻¹. When it came to I, however, soil-applied potassium iodate was only efficient in raising I concentration in cabbage leaves; biofortification of the other crops was not feasible. Both the single application and the combination application of micronutrient fertilizers resulted in similar increases in Se, Zn, and I content. We deduced from the findings of this study that agronomic biofortification of edible sections of different food crops with Zn, Se, and I can be a useful method to raise micronutrient concentrations, and the usefulness relies on crop species, fertilizer types, and application techniques.

Ali et al., (2019) performed pot research to assess the effectiveness of foliar spraying different concentrations (0, 50, 75, and 100 mg/L) of Zinc Oxide Nanoparticles (ZnO NPs) alone or in combination with biochar (1.0% w/w) on the Cd content in rice plants growing on an old Cd-polluted soil. According to the findings, ZnO NPs alone or in combination with biochar increased rice plant biomass and photosynthetic activity, decreased Cd concentration, and increased Zn concentrations in shoots and roots. The Cd level in rice shoots and roots was dramatically reduced by 30% and 31%, respectively, by foliar spraying with 100 mg/L ZnO NPs. After 100 mg/L ZnO NPs were combined with biochar, the Cd levels in rice shoot and root decreased by 39% and 38%, respectively. The pH of the soil was raised from 8.03 to 8.23 units by the ZnO NPs and biochar. The amendments placed over the control resulted in a considerable decrease in soil AB-DTPA-extractable Cd. The study came to the conclusion that rice plants may be grown using a foliar spray of ZnO NPs and biochar, particularly in regions with high Cd concentrations and high Zn deficiency.

Jalal et al., [25] in Peshawar, Khyber Pakhtunkhwa, Pakistan, conducted a study in the winter of 2016–2017 to investigate the effects of foliar zinc rates (0.1, 0.2, 0.3 and 0.4% ZnSO₄.7H₂O) and foliar iron rates (0.5, 1, 1.5 and 2% FeSO₄.7H₂O) on wheat physiology, phenology, yield indices, and grain Zn and Fe contents. According to research results, varied foliar Zn and Fe levels significantly increased grain Zn and Fe contents while also increasing leaf area per tiller, flag leaf area, productive tillers, yield components, biomass yield, and grain Zn and Fe contents. Grain yield was higher in the plants fertilized with 0.3% Zn and 1% Fe. Plants fertilizer with foliar Zn at 0.4% and Fe at 0.5% showed higher grain Zn content. The plants fertilized with 0.1% Zn and 2% Fe had higher grain Fe content. As a result, it can be said that plants fertilized with 0.3% foliar Zn and 1% Fe had enhanced yield components and yield, whereas plants fertilized with 0.1% Zn and 2% Fe had improved wheat grain quality (Zn and Fe content). In order to increase wheat yields and improve

grain quality under the study's edaphoclimatic conditions, foliar fertilization with 0.3% Zn and 1% Fe is advised.

Pal et al., [26], did a study to see how Ferrous Sulphate (FeSO₄), Zinc Sulphate (ZnSO₄), and Urea affected the biofortification of chickpeas. All On the bean plant, each of the three fertilizers was used in similar amounts, and the three were mixed together. The treatments were done when the plant was blooming and making pods. On the other hand, the same amount of beans was growing with and without special care. Similar Zn (44.01 and 43.01 mg Zn kg⁻¹ grain in the first and second year of study) and Fe (71.08 and 73.91 mg Fe kg⁻¹ grain) were obtained following foliar application of ZnSO₄ (0.5%), FeSO₄ (0.5%), and Urea (2%) in a tank mixture, demonstrating a relatively positive effect compared to the case of individual application of these nutrients (45.08 and 45.00 mg Zn kg⁻¹ and 47.58 and 48.66 mg Fe kg⁻¹ grain). Pouring with 0.5% ZnSO₄ and 0.5% FeSO₄ did not significantly increase Zn and Fe levels, but adding 2% Urea did. Tank mixes and individual applications of these nutrients resulted in significantly higher grain yields and protein concentrations than the application alone and the control. Chickpeas may have their Zn and Fe levels boosted by applying a tank mix of ZnSO₄ (0.5%), FeSO₄ (0.5%), and Urea (2%) throughout the growth and pod-forming stages. Focusing on this innovative biofortification strategy is crucial in the fight against world hunger.

As discovered by Stengolis and Nez in 2023, bio fortification is a sustainable method of providing more Iron (Fe) and Zinc (Zn) to those who need it. Cultivars with high Fe and Zn levels have been developed thanks to the efforts of the CGIAR and NARS, plant breeding programs, and new bio-enhanced varieties have been introduced with relative ease. A recent study has helped us learn more about how Fe and Zn are kept in balance and how genes are controlled. This has led to the discovery of potential genes for marker-assisted selection. Over the years, there has been more international cooperation between farming and food groups in creating and using different application methods and relationships. More and more proof shows that bio-enriching foods with Fe and Zn work and the effects look good. While bio fortification is gaining popularity, more work has to be done to achieve the larger goals of assuring food security and eliminating the "hidden hunger" caused by a shortage of iron and zinc.

According to Praharaj et al. [28], vitamin deficiency is a significant issue for people's health worldwide. More than two billion individuals throughout the globe are deficient in vitamin intake. Low zinc solubility, adverse health impacts, and widespread Zinc (Zn) shortage in soils have prompted scientists to consider low-cost, long-term solutions to this problem. Even though they work, tactics like adding zinc to food and taking supplements are too pricey and out of reach for most people in low-income countries who are most likely to have a zinc shortage. In this case, adding zinc to essential food items through bio fortification is a good idea. Approaches to agricultural bio fortification that use crop reproduction with Zn-based manures at the correct times are cost-effective, easy to use, and effective ways to add zinc to grains. Even though it takes a long time, genetic bio fortification works very well. Once set up, Zn-enriched genotypes can also be used for many years without any extra costs. Genetic and farming bio fortification can be beneficial ways to deal with the Zn shortage.

Vitamin deficiency is a major global health problem, say

Praharaj et al. [28]. Around the world, vitamin deficiencies affect over two billion people. Scientists are looking for low-cost, long-term solutions to the widespread Zinc (Zn) deficit in soils, which has led to poor zinc solubility and adverse health effects. Getting rid of vitamin deficits and making more food available is done in several ways. These include:

- Changing what people eat
- Adding vitamins and minerals to food in factories and through food additives
- Using farming techniques like fertilizing the soil, bioaccumulation, and crop rotation
- Using bioengineering like gene editing and plant breeding to make food more healthy

When it comes to the acceptance of new enriched crops, these projects should consider the eating habits and food preferences of customers and stakeholders. Zn and Fe shortages are often caused by lousy soil nutrition, which means these elements must be present in more amounts or are hard to get to in food crops like common beans. The mechanisms by which common beans accumulate Fe and Zn are reviewed here. The importance of common beans in Africa's food supply cannot be overstated. Here, we closely examine the conventional plant breeding, transgenic, and gene editing techniques that have been used to increase Fe and Zn storage in legumes. We also discuss the factors contributing to the success of a bio fortification program for beans, as well as information gaps, potential remedies, and prospects.

Rahman et al. [30] says that people in poor countries are much more likely to get sick because they don't get enough vitamins like zinc. Many people in Pakistan (22.1% of the population) don't get enough Zinc (Zn) because they don't eat enough zinc-rich foods. This is especially true for women and children (under five years old). Wheat is a staple food in Pakistan, but it has little Zn that the body can use. But in the last ten years, there have been more people who need more food. This is because more people are aware of the problem, zinc fertilizers are being used quickly, more national and international research projects on zinc biofortification in significant crops, and more people have access to food. But many people need more zinc or other minor elements. Multiple studies show that people who eat enriched wheat flour get more zinc daily than those who don't (Zincol-2016). This means that bio fortification methods could reduce the risk of zinc shortage in rural and poor areas by a significant amount. The zinc fertilization method helped increase the amount of zinc in the grain and increase the number of grain yields and economic gains. Also, Zn-enriched seeds showed a lot of natural protection from abiotic stress and a high grain output in many different climates. But many things, like soil, climate, genetic variation, food content, social factors, etc., make it hard for biofortification efforts. This review discusses the zinc shortage, how agriculture works, and how well genetic biofortification works in Pakistan. The finances of agricultural bio fortification and the cost-effectiveness of zinc fertilization in Pakistan are also discussed. So is the ability of zinc-rich seeds to deal with environmental stress. It also calls attention to the problems that make it hard to keep working on bio fortification.

Obaid et al., [31], look at Zinc (Zn) fertilizer control methods and breeding efforts to make high-quality corn for people to eat. It is crucial for people's health that high-yield corn types with high zinc levels in grains are bio fortified with zinc. A lit-

erature study was done on choosing maize types with better traits to increase zinc output, zinc content, and zinc availability in maize plants grown on zinc-deficient soils. The things that affect how Zn moves from the earth to plants and people are found and looked into. Many people worldwide, especially those who eat a lot of corn, have health problems because they don't get enough zinc. The lack of zinc in the soil could be fixed with a farming method. Because there are many kinds of crop fertilizer, the type and when it is put on each corn plant are very important for the best crop. Clinical tests have shown that genetically enriched corn makes it easier for the body to absorb zinc. Both farming and breeding methods for bio fortifying corn show promise for ensuring that people get enough zinc for their bodies to work well.

Cakmak et al. [32] said that Zinc (Zn) is still a significant public health issue in developing countries because people don't get enough of it in their diets. The leading cause of this problem is that many people eat cereals that are low in zinc and don't absorb it well. Modern grains naturally have very little zinc in them, so they can't meet people's zinc needs. Today, up to 50% of wheat lands worldwide are thought to have too little zinc. Bio fortification techniques are used in agriculture to make plants more nutritious. Genetic bio fortification and agricultural bio fortification are two examples. Genetic bio-fortification relies on conventional plant breeding and genetic engineering to increase the nutritional content. Agricultural bio fortification is based on making the best use of fertilizers. This review is about zinc biofortification in agriculture, which is helpful in other grain crops like wheat and rice. To find problems with adding zinc to food crops through bio fortification, molecular and genetic studies of how zinc is taken up, moved, and stored in grains are needed. Transgenic plants with high zinc levels in their seeds are often tested in a controlled lab or garden where there is enough zinc in the growing medium for the plants to use. But they couldn't always work at their best in "real" settings because Zn was hard to get, and there were a lot of stresses like drought. If the number of things that can be moved and stored is limited, what good is a modern method for moving and storing items? It is essential to give agricultural plants enough zinc through the soil and foliar fertilization in the field because the amount of zinc needed to have a noticeable effect on human health is much higher than the amount required stopping yield loss from zinc deficiency.

According to White et al. [33], over a third of the global population does not get enough zinc in their diets. Increasing zinc intake via diet and bio-supplementing food crops with zinc may help remedy this problem. Using zinc fertilizers and developing crop genotypes that collect more zinc from the soil and store it in edible parts are examples of bio-fortification methods. Zinc fertilizers may boost the zinc content of your plant's roots, leaves, and stems. Zinc fertilizers have been shown to increase zinc concentrations in plant tissues by as much as 500–5,000 mg kg⁻¹ dry matter in the hearts and 100–700 mg kg⁻¹ dry matter in the leaves without negatively impacting crop output. Applying zinc sulfate or zinc oxide as a foliar fertilizer may increase zinc levels in non-woody shoot tissues. Zinc is so immobile in the phloem that it is seldom found in quantities higher than 30–100 mg/kg in fruits, seeds, and tubers. However, it is conceivable to boost these phloem-rich tissues physiologically by utilizing genetically modified plants with improved phloem zinc transport capacity. Additionally, genetically engineered plants with greater tolerance to high tissue zinc concentrations may increase zinc levels in all meals and maximize dietary zinc absorption.

According to Szerement et al. [34], micronutrient insufficiency in humans is a significant global issue, especially for those diets (mostly plant-based) are deficient in essential vitamins and minerals. It concerns the low concentration and poor soil bioavailability of trace elements and abiotic stressors that prevent plants from growing and developing correctly. Agronomic crop biofortification is a possible way to raise the concentration of micronutrients in edible parts of crops without harming the harvest. It is often regarded as the most cost-effective means of solving hidden hunger worldwide. Considerations (including application technique, trace element kind and dosage, bio and Nano fertilizers, and other factors) that influence the success of enhanced crops are analyzed. Zinc, selenium, and iron accumulation in human food crops; their effects on crop productivity; their impact on crop morphology and metabolism; and their effects on plant resistance to abiotic stress, including salt, severe temperature, and heavy metals. Finally, some thoughts on where we may take research into biofortification in agriculture in the future are shared.

De Valenca et al. [35] suggests that agronomic biofortification, which involves adding mineral micronutrient fertilizers to plant leaves or soil to boost the micronutrient content of edible crop portions, may help alleviate hidden hunger. There is inconclusive evidence that agricultural bio-fortification promotes human health, even though it may boost essential crops' production and nutritional content. Integrated soil fertility management is necessary because micronutrient fertilization is most successful when used with NPK, organic fertilizers, and improved crop varieties. Even though genetic bio-fortification may be more cost-effective in the long run, agronomic biofortification offers a rapid and efficient technique to boost the concentration of micronutrients in food crops.

In 2022, Dhaliwal et al. highlighted bio-fortification as an agricultural method to boost crop productivity and address food security concerns. This dissertation examines many novel and distinctive bio-fortification strategies for enhancing the nutritional content of field crops, such as grains, legumes, oil seeds, and fodder crops. Composted soil and foliar fertilizers are two agronomic methods that boost crop micronutrient density. Traditional breeding strategies for bio-fortification rely heavily on selecting efficient genotypes or crossing plants with the necessary nutritional qualities without compromising agricultural and commercial outputs. On the other hand, transgenic/biotechnological procedures use the creation of transgenes to move micronutrients across tissues to increase their bioavailability repeatedly. Soil microorganisms grow the number of nutrients that are accessible in the rhizosphere via a variety of mechanisms, including synthesis, mobilization, transformation, and the production of siderophores, which further aid plants in absorbing minerals. Diverse sources of trace elements, such as chelates, nanoparticles, and mineral solutions, are essential to the bio fortification process because they regulate plants' rate and mode of absorption. Bio fortification has raised agricultural yields and met quality requirements to solve hidden hunger, proving that it is a workable and inexpensive solution. To guarantee food security and high nutritional quality for humans and cattle, this review article provides researchers with information on the correct potential of bio fortification to boost crop and pasture production with extra nutrients.

According to Anwar et al. [37], the hidden hunger that affects over two billion people worldwide presents a new challenge for scientists, particularly those in the agriculture business. This is-

sue is more common in developing nations since impoverished people have less money to spend on food. Among the strategies used to lessen the effects of malnutrition, agricultural bio-fortification is the most effective in raising the micronutrient content of grains. Maize yield and grain quality were studied concerning the application of Zinc Sulfate ($ZnSO_4$) and Iron Sulfate ($FeSO_4$) in a field experiment. Maize yield and grain quality were enhanced by applying $ZnSO_4$ and $FeSO_4$ to the soil or plant leaves. After spraying $ZnSO_4$ (10 kg ha⁻¹), crop yield and plant height were maximized. Co-application of 10 kilograms of Zinc Sulfate ($ZnSO_4$) per hectare (ha⁻¹) and 12 kilograms of Iron Sulfate ($FeSO_4$) per Hectare (ha⁻¹) increased ear grain, 1000 grain weight, biological yield, and grain yield. $ZnSO_4$ at 0.1% and $FeSO_4$ at 0.3% were found in the highest chlorophyll-containing leaves. The zinc and iron content of the grain improved when its leaves were treated with 0.1% $ZnSO_4$ and 0.3% $FeSO_4$. Combining 10 kg ha⁻¹ $ZnSO_4$ and 12 kg ha⁻¹, $FeSO_4$ increased yield, whereas using 0.1% $ZnSO_4$ and 0.3% $FeSO_4$ as foliar sprays enhanced quality. Foliar spraying is typically favored to improve development, growth, production, and grain quality.

Hafeez et al. [38] tried to lessen the severity of hidden hunger (micronutrient deficiency) throughout the 2017–2018 and 2018–2019 wheat growing seasons by agricultural bio-fortifying two wheat varieties with wheat bran and iron. Spring-sown bread wheat varieties Zincol-16 (Zn-efficient with high yield) and Anaz-17 (Zn-inefficient with high gain) were treated with either processed zinc (10 t/ha) or iron (12 t/ha) or their combination, and their growth traits, productivity, and quality were measured. No inert substance, such as zinc, iron, or a mixture, was employed. The grain's maximum levels of zinc and iron have been raised due to their sole usage. After adding Zn and Fe, the bread already had a significant quantity of Ca. To increase the starch content, he just needed to be engaged once. Wet gluten may be reduced by using either zinc, iron, or both. In the untreated group, Anage-17 had the best protein. Wet gluten, starch concentration, and ionic strength were all the better in Zincol-16 than in An Age-17. Yield and growth metrics were considerably improved by combined treatment compared to single applications of Zn or Fe (p 0.05). According to the maximum yield index, the combination treatment also produced the highest grain and organic matter yields. It was shown that the Anage-17 variety was much less robust in terms of both growth and creation. The highest result of bread wheat is achieved by mixing Zn and Fe, which also improves the grain quality (more Zn, Fe, Ca, starch, and reduced gluten content).

Methodology

Finding a single conclusion about a topic of research with multiple narratives is a challenging and perplexing task. In this situation, review research aids in providing an objective methodology-based summary of numerous published outcomes. This work is using the methodology to give a systematic review on zinc biofortification effect on crops and its effectiveness in preventing zinc malnutrition.

For this study, we conducted a survey of previously published researches on the topic of "zinc biofortification effect on different crops", "biofortification of zinc effect on crop qualitative traits and quantitative traits" and its effectiveness in preventing zinc malnutrition. All available data or researches on biofortification, zinc biofortification and effect on crops quality and other physical traits and on its nutritious value and its effectiveness in decreasing zinc malnutrition was systematically collected and analyzed. For this study, a comprehensive search was

done on "Google", "Google advanced search", "Google Scholar", "PubMed" and Sci-Hub using a keyword "Zinc deficiency" and "Zinc deficiency in Pakistan" "Zinc biofortification" "Zinc", Zinc biofortification, Foliar application of zinc, Zinc deficiency, Zinc bioavailability", Zinc biofortification effect on crops. We analyzed all the studies containing these words and then detailed search were done by using cross references and bibliographies of already available data and publications to increase the pool of our studies.

After collecting all the data, the results were analyzed and a systematic review was written on it.

Results and Discussions

Soil of KPK and Punjab is deficient in several major nutrients i.e. iron and zinc. The plant grown in that soil is also deficient in those nutrients. The strategy used to eliminate that deficiency is biofortification. There are different types of biofortification methods but agronomic biofortification is most commonly used and has a very positive effect on crops.

Biofortification Effect on Different Crops

We collected a total of 26 studies which showed zinc alone or biofortified with other nutrients effect on different crops i.e. wheat, rice, barley, cereals, and corn etc. and also showed zinc bioavailability in these crops.

Their results show a positive impact in terms of zinc concentration in their yield and quantity of yield is also increased.

Through biofortification we can increase crops yield and nutritional value. Which is an affordable strategy to eliminate hunger and hidden hunger issues in Pakistan.

[13] look into how wheat's yield qualities and nutritional value are impacted by Iron (Fe) and Zinc (Zn). The treatment that used 0.3% Fe sprays produced the best yield (4.9 t ha⁻¹) and raised the amount of Fe in grains by 31% compared to the control. The Zn concentration in grains increased by 43.8% in the mean treatment compared to the control, after receiving 0.3% Zn spray. When these nutrients were applied to the soil, encouraging outcomes were also shown in comparison to the control, but foliar sprays had a more substantial impact on the number of tillers, spikelets per spike, and grains. We may not only boost wheat productivity but also address the major problem of hunger that exists today, especially in underdeveloped countries.

The results showed that when nutrients (Ca at 3% + Zn at 2% + Fe at 1%) were applied together, the plants' height (30.0%), number of leaves (53.6%), stem diameter (48.6%), leaf area per plant (77.2%), fresh biomass (48.2%), dry matter yield (120.8%), dry matter contents (49.1%), crude proteins (78.6%), and ash contents (120.8%) were all increased in comparison to the control (no spray). However, the control treatment yielded the highest However, the control treatment yielded the highest Acid-Detergent Fiber (ADF) and Neutral Detergent Fiber (NDF) concentrations. The highest plant Zn concentrations were Ca at 3%, Zn at 2%, and Fe at 1% Asif et al., [14].

Ramzan et al., [15] the height of plants has increased when Zinc Sulphate (ZnSO₄) and Iron Sulphate (FeSO₄) were applied through soil and foliar application and as alone or in combined form. The combined foliar spray of 0.5% ZnSO₄ and 1% FeSO₄ considerably increased the maximum growth or quality features of wheat when compared to other Zn and Fe concentrations applied. In comparison to soil application, foliar treatment is more

suitable for making nutrients available to plants for optimum growth. It is advised to apply Zn and Fe together (0.5% ZnSO₄ and 1% FeSO₄) by foliar spray to increase wheat crop productivity and grain quality.

According to the findings of this study, the highest grain (3.46 t/ha) and straw (7.93 t/ha) yield of basmati rice was obtained applying 4.0% Zn-coated urea (ZnCU) + 0.2% Zn foliar spray (FS) using ZnSO₄ • 7H₂O. Zinc coated urea increased rice productivity by about 25.4% on average when compared to Commercial Urea. According to this study, Zinc coated urea would be a crucial, low-cost, and practical technique for raising yield, N Usage Efficiency (NUE), and biofortification, as well as for reducing the problems caused by Zn deficiency in the human diet in many poor economies Bana et al., [16].

Applying ZnBTU (Zinc (Zn), Boron (B), Thiourea (TU)) to the leaves of delayed-sown wheat throughout the grain filling and booting stages may be a potential way to boost grain output Ahmad et al., [18].

Zn application boosted maize grain output and Zn content when applied as a soil or foliar application. The study came to the conclusion that using zinc fertilizer is an effective way to increase corn output, grain concentration, and eventually corn quality Kirtika et al., [19].

The molar ratio of phytates decreased; however, zinc did not significantly increase zinc bio accessibility. These results can be used to develop wheat varieties that are higher in micronutrients and have better nutritional characteristics Jiang et al., [20].

Rice genotypes' average Zn bioavailability increased by a factor of 2.06 on average. Zinc application also increases crop production, Zn content, and bioavailability zinc in rice. Agronomic Zn biofortification of rice may be a simple, flexible, and quick strategy to increase crop yield Akram et al., [21].

Ciriello et al., [22] increasing the concentration of Zn in the nutrient solution significantly reduced the yield, this reduction was less evident in basil cultivars 'Aroma 2'. The study's findings showed that the yield was greatly decreased when the concentration of Zn in the nutrient solution was raised; this loss was less pronounced in "Aroma 2." But irrespective of cultivar, the application of the maximum Zinc dose (50 M) raised the concentration of carotenoids, polyphenols, and antioxidant activity on average by 19.76, 14.57, and 33.72%, respectively, when it comes to Zn, soil-applied zinc sulphate was only shown to be beneficial in bio fortifying cabbage leaves and canola seeds, boosting their Zn concentrations to 35 and 61 mg kg⁻¹, respectively. However, foliar zinc sulphate application was successful in biofortifying winter wheat, increasing the grain's Zn content from 20 to 30 mg kg⁻¹. When it came to iodine, however, soil-applied potassium iodate was only efficient in raising I concentration in cabbage leaves. Biofortification of the other crops was not feasible Mao et al., [23].

Rice plants may be grown using a foliar spray of zinc oxide nanoparticles (ZnO NPs) and biochar, particularly in regions with high Cadmium (Cd) concentrations and high Zn deficiency Ali et al., (2019). In order to increase wheat yields and improve grain quality under the study's edaphoclimatic conditions, foliar fertilization with 0.3% Zn and 1% Fe is advised Jalal et al., [25].

Chickpeas may have their Zn and Fe levels boosted by applying a tank mix of Ferrous sulphate ZnSO₄ (0.5%), Zinc sulphate FeSO₄ (0.5%), and Urea (2%) throughout the growth and

pod-forming stages. Focusing on this innovative biofortification strategy is crucial in the fight against world hunger Pal et al., [26].

As discovered by Stengolis and Nez in 2023, bio fortification is a sustainable method of providing more Iron (Fe) and Zinc (Zn) to those who need it. Cultivars with high Fe and Zn levels have been developed thanks to the efforts of the CGIAR and NARS, plant breeding programs, and new bio-enhanced varieties have been introduced with relative ease. More and more proof shows that bio-enriching foods with Fe and Zn work and the effects look good. While bio fortification is gaining popularity, more work has to be done to achieve the larger goals of assuring food security and eliminating the "hidden hunger" caused by a shortage of iron and zinc.

Approaches to agricultural bio fortification that use crop reproduction with Zn-based manures at the correct times are cost-effective, easy to use, and effective ways to add zinc to grains. Even though it takes a long time, genetic bio fortification works very well. Once set up, Zn-enriched genotypes can also be used for many years without any extra costs. Genetic and farming bio fortification can be beneficial ways to deal with the Zn shortage Praharaj et al. [28].

Effectiveness of Biofortification in Eliminating Zinc Deficiency

Biofortification is an effective and economically affordable method through which we can increase the grain zinc content.

It also increases crop productivity and these crops are better able to adopt environmental stress. So, in this way we can eliminate the hunger issue in Pakistan.

Biofortification also reduces phytate content in crops and increases zinc bioavailability.

These crops have better dietary zinc bioavailability than traditional ones.

In Pakistan, zinc deficiency has decreased i.e 22.1% in women and 18.6% in children than 2011 National Nutritional Survey which is 47.6% in pregnant and 41.3% in non-pregnant women and in children zinc deficiency is 39.2%.

This is due to multiple public health awareness and training of farmers and also biofortification of staple crops.

Rahman et al. [30] says that people in poor countries are much more likely to get sick because they don't get enough vitamins like zinc. Many people in Pakistan (22.1% of the population) don't get enough Zinc (Zn) because they don't eat enough zinc-rich foods. This is especially true for women and children (under five years old). Zinc fertilizers are being used quickly, more national and international research projects on zinc biofortification in significant crops, and more people have access to food. But many people need more zinc or other minor elements. The finances of agricultural bio fortification and the cost of using zinc fertilization in Pakistan is also less. So, the ability of biofortified zinc-rich seeds to deal with environmental stress is also better. Many things, like soil, climate, genetic variation, food content, social factors, etc., make it hard for biofortification efforts.

The lack of zinc in the soil could be fixed with a farming method. Because there are many kinds of crop fertilizer, the type and when it is put on each corn plant are very important for the

best quality. Clinical tests have shown that genetically enriched corn makes it easier for the body to absorb zinc. Both farming and breeding methods for bio fortifying corn show promise for ensuring that people get enough zinc for their bodies to work well Obaid et al., [31].

Bio fortification techniques are used in agriculture to make plants more nutritious. Genetic bio fortification and agricultural bio fortification are two examples. Genetic bio-fortification relies on conventional plant breeding and genetic engineering to increase the nutritional content. Agricultural bio fortification is based on making the best use of fertilizers. Transgenic plants with high zinc levels in their seeds are often tested in a controlled lab or garden where there is enough zinc in the growing medium for the plants to use. It is essential to give agricultural plants enough zinc through the soil and foliar fertilization in the field because the amount of zinc needed to have a noticeable effect on human health and then the amount required to stop yield loss from zinc deficiency Cakmak et al. [32].

Zinc fertilizers may boost the zinc content of your plant's roots, leaves, and stems. Zinc fertilizers have been shown to increase zinc concentrations in plant tissues by as much as 500–5,000 mg kg¹ dry matter in the hearts and 100–700 mg kg¹ dry matter in the leaves without negatively impacting crop output. Additionally, genetically engineered plants with greater tolerance to high tissue zinc concentrations may increase zinc levels in all meals and maximize dietary zinc absorption White et al. [33].

Agronomic crop biofortification is a possible way to raise the concentration of micronutrients in edible parts of crops without harming the harvest. It is often regarded as the most cost-effective means of solving hidden hunger worldwide. Considerations (including application technique, trace element kind and dosage, bio and Nano fertilizers, and other factors) that influence the success of enhanced crops. Zinc, selenium, and iron accumulation in human food crops; their effects on crop productivity; their impact on crop morphology and metabolism; and their effects on plant resistance to abiotic stress, including salt, severe temperature, and heavy metals are given in this research. Szerement et al. [34].

De Valenka et al. [35] suggest that agronomic biofortification, which involves adding mineral micronutrient fertilizers to plant leaves or soil to boost the micronutrient content of edible crop portions, may help alleviate hidden hunger. There is inconclusive evidence that agricultural bio-fortification promotes human health, even though it may boost essential crops' production and nutritional content. Integrated soil fertility management is necessary because micronutrient fertilization is most successful when used with NPK, organic fertilizers, and improved crop varieties.

According to Anwar et al. [37], the hidden hunger that affects over two billion people worldwide presents a new challenge for scientists, particularly those in the agriculture business. This issue is more common in developing nations since impoverished people have less money to spend on food. Among the strategies used to lessen the effects of malnutrition, agricultural bio-fortification is the most effective in raising the micronutrient content of grains. Maize yield and grain quality were studied concerning the application of Zinc Sulfate (ZnSO₄) and iron sulfate (FeSO₄) in a field experiment. The grain's maximum levels of zinc and iron have been raised due to their sole usage. After adding Zn and Fe, the bead already had a significant quantity of Ca. To in-

crease the starch content, he just needed to be engaged once. Wet gluten may be reduced by using either zinc, iron, or both. In the untreated group, according to the maximum yield index, the combination treatment also produced the highest grain and organic matter yields. It was shown that the Anage-17 variety was much less robust in terms of both growth and creation.

Conclusion

The goal of the current systematic review was to evaluate the impact of zinc biofortification on health and performance in people. Their findings demonstrate that zinc concentration has a favorable impact on yield and also raises yield. In comparison to the control treatment, zinc-enriched seed boosted wheat grain yield and improved its quality in different crops experiments. Using zinc-enriched grains as seed in the upcoming growing season can cost-effectively increase crop yield because zinc-rich grains are a byproduct of zinc biofortification. The widespread Zinc (Zn) shortage in soils, inadequate zinc absorption in the human diet, low bioavailability, and health effects have pushed the research community to explore for an affordable and long-term solution to minimize zinc deficiency.

The approach of agrobiologically enrichment, which entails timely delivery of zinc-based fertilizers to crops, is affordable, simple to administer, and extremely effective for ensuring zinc enrichment of cereal crops. Gene bio amplification takes a lot of time, but it also has great effectiveness. Food crops' nutritional value and tolerance to water shortages can both be improved through agrobiome enrichment with zinc. Zinc can improve the physiological and enzymatic antioxidant defense mechanisms of plants, increasing their resilience to water stress. An efficient and affordable way to raise the zinc content of grains is biofortification. Additionally, it enhances the functionality of items and increases their resistance to environmental factors. Pakistan's hunger issue can be resolved in this way. Additionally, biofortification raises the bioavailability of zinc and lowers the phylate concentration of crops. A significant barrier to agricultural productivity and a known issue with human health is soil Zinc (Zn) deficiency. The biofortification enrichment with zinc that has been demonstrated to be very successful for rice, as well as other cereal crops, is the main topic of this paper.

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