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Review Article

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Nano-Biofortification of Millets: A Review

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Abstract

The world's population faces nutritional insecurity due to a reliance on grain-heavy diets low in micronutrients. In drought-prone regions of Asia and Africa, millets are a crucial energy source, rich in proteins, vital amino acids, minerals, and vitamins. Biofortifying staple crops is a cost-effective strategy to combat micronutrient deficiencies, particularly in impoverished areas where access to supplements is limited. While the green revolution boosted agricultural output, it often overlooked the nutritional quality of food. Biofortification using nanoparticles and nanomaterials can enhance nutrient delivery and improve crop health without significantly harming the environment. Micronutrient-enriched nano fertilizers can increase yields and nutritional quality, especially for zinc and iron. Studies indicate that nano-fertilizers improve nutrient utilization, reduce soil toxicity, and decrease application frequency, making nanotechnology a promising solution for sustainable crop production, especially in developing nations.

Keywords: Low Micronutrients, Millets, Biofortification, Nanoparticles, Nutritional quality, Nano-fertilizers.

1. Introduction

World Health Organization (WHO) defines micronutrients as compounds required in < 100 mg/d, which includes vitamins (vitamin A, B_9) and minerals such as iron (Fe), zinc (Zn), and iodine (I). Disease caused by the deficiency of these micronutrients in humans is known as 'Micronutrient Malnutrition' or 'Hidden Hunger', which causes early death, impaired health, vision effects, mental disorders, learning impairment, improper growth, and lassitude. According to the World Health Organization (WHO), 45% of deaths in children below 5 years of age are due to malnutrition[1].

In developing countries, hidden hunger or micronutrient deficiency is of grave concern because it contributes to about 0.5% of deaths in India in 2016. About 42% of children <5 yrs. and 40% of pregnant women are anemic due to iron deficiency, referred to as iron deficiency anemia (IDA) [2]. About 24.8% of the world's population suffers from iron deficiency[3]. National Family Health Survey- 4 showed that India has the highest percentage of anemia worldwide. Iron deficiency in children is about 58.6%, in non-pregnant women is about 53.2%, and in pregnant women about 50.4%[4]. Zinc deficiency can lead to death from various diseases. In humans, it affects multiple systems, including the immune, skeletal, reproductive, gastrointestinal, neurological, and intestinal systems.

Consequently, zinc deficiency can present itself in several ways, with the most common being increased rates of pneumonia, malaria, diarrhea, and other illnesses.

The government has taken many initiatives against micronutrient deficiency, but the problem still exists in a large population. Food fortification, dietary diversification, nutritional education, micronutrient supplements, and maintenance of environmental sanitation and hygiene are various ways to overcome the problem.

Food fortification is one of the best ways to tackle the problem in all age groups. Enrichment of food with micronutrients can be achieved by biofortification of staple food crops.

Biofortification is a way to increase the micronutrient content in food crops. It can be achieved by various methods, such as conventional plant breeding, transgenic approaches, agronomic approaches, and Nanobiofortification.

Conventional breeding: utilizes the genetic variation present in different gene pools of the target crop.

Transgenic approach: A genetically engineered plant is made by altering the gene segment.

Agronomic approach: by the application of the nutrient-containing fertilizers either by foliar spray or by soil application.

Nanobiofortification: Application of the Nano-fertilizers to ensure target-bound slow delivery of nutrients to plants, reduce nutrient volatilization, reduce nutrient leaching, and increase bioavailability of nutrients.

India is the leading producer of millet, accounting for about 80% of the global millet production[5]. Millets are also known as 'small seeded plants' which include Pearl millet (*Pennisetum glaucum* L.), Finger millet (*Eleusine coracana* L.), Foxtail millet (*Setaria italica* L.), Proso millet (*Panicum miliaceum* L.), Barnyard millet (*Echinochloa* spp.), Kodo millet (*Paspalum scrobiculatum* L.), and Little millet (*Panicum sumatrense* L.). Among all millets, about 95% are pearl millets (*Pennisetum glaucum* L.) [6, 7, 8]. The sixth largest production is finger millets (*Eleusine coracana* L.), which serve as the primary food for the rural population of east and central Africa and southern India[9]. Millets are dominant over rice and wheat due to their high nutritional quality, they contain high amounts of protein, dietary fibers, iron, zinc, calcium, phosphorus, potassium, and vitamins. B and some essential amino acids[10].

Millets also have some antinutritional compounds like phytates, polyphenols, and tannins that reduce the absorption of multivalent cations like Fe^{2+}, Zn^{2+}, Ca^{2+}, Mg^{2+}, and K^{+}[11]. The anti-nutritional compound can easily be removed by decortication, malting, fermentation, roasting, flaking, and grinding. 80% of millet is used as food, and the rest is used as fodder and in the brewing industry[12]. Millets are a super food for infants, lactating mothers, senior citizens, and convalescents. Millets are considered 'gluten-free' as they release sugar very slowly into the bloodstream[13]. As millets are rich in protein and dietary fibers, they are recommended for people suffering from diabetes and cardiovascular disease[14]. They are rich in flavonoids and phenolic acids, which help fight against free radicals generated by oxidative stress and lower blood sugar levels[15].

Among all millets, pearl millets have the highest content of iron, zinc, and lysine (17-65 mg/g of protein). Finger millets have high levels of Ca that help to strengthen bones, K that helps to prevent diabetes, renal and cardiovascular diseases, Mg, and some essential amino acids like Met, Lys, Trp, and polyphenols[10].

The emergence of green nanotechnology has led to significant interest in this field among global scientific researchers. Nano-materials harmful effects can be minimized by implementing green nanotechnology, which is an effective means of reducing these risks[16]. Nanotechnology has emerged as one of the most active areas of study[17].

Green chemistry employs chemical principles to minimize or eliminate the use of hazardous substances, leading to a significant decrease in toxic residues that are harmful to both humans and the environment. Green synthesis is considered a feasible method for nanoparticle synthesis because it is biocompatible, unrestricted, and environmentally friendly.

To prepare nanoparticles, the most efficient method is green synthesis, which minimizes toxic substances and enhances stability while being eco-friendly and cost-effective. Both environmental and biomedical contexts favor green synthesis methods as a more effective approach[18]. The phytochemical compounds found in plants include phenols, terpenoids, polysaccharides, and flavonoids that possess redox properties. Thus, they are advantageously used for the green synthesis of nanoparticles.

Compared to chemical and physical methods, green synthesis has several advantages, such as being non-toxic[19], environmentally friendly[20], eco-friendly, cost-effective[21], and more sustainable[22]. The process of biogenic reduction of metal precursors to corresponding NPs is advantageous for its environmental impact[23], durability[24], chemical safety[25], affordability[26], and mass production[27].

2. Significance and Nutrient Value of Millets

2.1. Staple Food:

Millions of rural poor people in developing countries rely on millets as their primary food source. Millets are among the oldest food crops known to humankind and were the first cereal crops used for domestic purposes. Instead of belonging to a specific taxonomic group, they are categorized by their functional or agronomic characteristics. Millets are exceptionally hardy and thrive in arid regions, making them increasingly popular as staple foods, particularly in large areas of India and sub-Saharan Africa. Their high productivity and short growing season in dry and hot conditions contribute to their desirability. Due to their nutritious content, millets are now often referred to as "Nutri-cereals."

A class of cereal crops is represented by millets. Major and minor millets are the two categories into which they are separated. Pearl millet and sorghum are the two main millets. Finger millet, Proso millet, Foxtail millet, Kodo millet, Small millet, and Barnyard millet are the minor millets. Millets are often referred to as "Small-seated grasses" and include various types such as pearl millet, finger millet, foxtail millet, proso millet, barnyard millet, kodo millet, and tiny millet. A substantial portion of millet production is made up of pearl millet. As a result, millets are commonly consumed in multigrain form to take advantage of their combined nutritional benefits.

Millets are native to many regions around the world, but they are believed to have evolved in tropical western Africa, where the highest diversity of both wild and cultivated varieties can be found. In East Asia, millets have been cultivated for over 10,000 years and have played a significant role as staple food crops throughout human history, particularly in Asia and Africa.

Millets are an important food source in resource-limited countries in Asia and Africa, with an average yearly production of 14.2 million tons and 12.4 million tons, respectively. They rank second in calorie content after cereal grains. As illustrated in Figure 1, India is the world's largest producer of millets, contributing approximately 491% of global output, as shown in Figure 2.

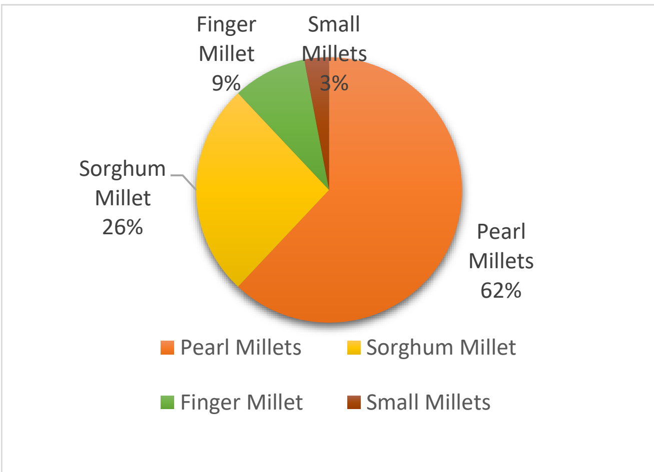

Figure 1. Production of Millet in India (2023-24) [Source: APDEA].

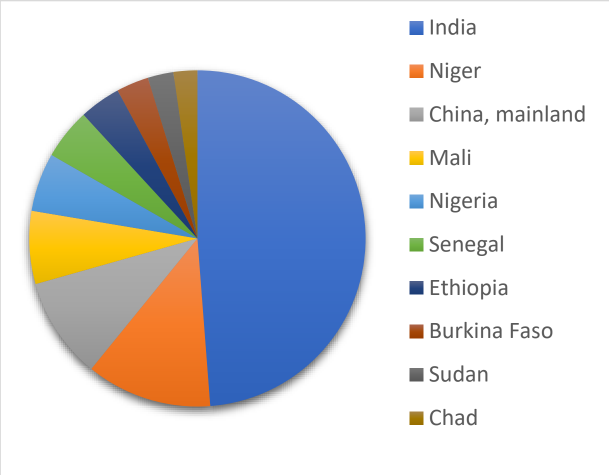

Figure 2. Global Production of Millets (Source: FAOSTAT-2023)

2.2. Nutritional Value:

Millets are an excellent source of protein, vitamins, and minerals, making them highly nutrient-dense. Approximately 80% of millet grains are used for food, while the remainder is used for animal feed and alcoholic beverages[10, 12]. Millets are recommended for the health of newborns, nursing mothers, the elderly, and individuals recovering from illness. These grains are considered gluten-free because they release sugar into the bloodstream gradually. Additionally, millets contain health-promoting flavonoids and phenolic acids, which play a crucial role in lowering blood glucose levels and preventing oxidative stress caused by free radicals. When compared to other millets, pearl millet has higher levels of iron (Fe), zinc (Zn), and lysine, ranging from 17 to 65 mg per gram of protein. Foxtail millet contains significant amounts of fat (4%) and protein (11%), with its protein composed of prolamins (39.4%), glutelins (9.9%), and albumins and globulins (13%). For these reasons, foxtail millet is recommended as an ideal diet for diabetics. Additionally, it boasts high concentrations of carotenoids, phenols, and phenolic acids, which may act as antioxidants[28]. Finger millet grains are particularly rich in minerals such as calcium, magnesium, and potassium[29]. According to Pettifor[30], adequate calcium intake is vital for maintaining healthy bones, while potassium may help delay the onset of diabetes, kidney issues, and heart problems. Barnyard millet is notable for its high concentrations of polyphenols[29] and essential amino acids such as methionine, lysine, and tryptophan[31]. According to Saleh et al.[10], barnyard millet is recognized as the best source of crude fiber (13.6%) and iron (186 mg/kg dry matter), while Proso millet boasts the highest protein content at 12.5%. Additionally, barnyard millet grains contain beneficial components like glucan and gamma-aminobutyric acid (GABA), which serve as antioxidants and help reduce blood lipid levels[32]. Given its low carbohydrate content compared to other millets, barnyard millet is recommended as an ideal grain for individuals with type II diabetes. The greatest advantage of Kodo millet is its high magnesium content (1.1g/kg dry matter). Therefore, millets are eaten as a multigrain to benefit from the combined health advantages.

Millets	Nutritional Value
Pearl Millets [*Pennisetum glaucum* (L.)]	Rich in iron (Fe), zinc (Zn), and lysine, containing between 17 to 65 mg of lysine per gram of protein, which is higher than that found in other millets. The total phenolic content in pearl millet has been reported to be 168 mg per 100 grams, with ferulic acid equivalents present in the soluble phenolic fraction. Additionally, the total flavonoid content in pearl millet is 49 mg per 100 grams, expressed in catechin equivalents within the soluble phenolic fraction[33].
Foxtail Millet [*Setaria italica* (L.)]	High amount of protein (11%) and fat (4%). The protein fractions are represented by albumins and globulins (13%), prolamins (39.4%), and glutelins (9.9%). It is thus recommended as an ideal food for diabetics. It also contains significant amounts of potential antioxidants like phenols, phenolic acids, and carotenoids[28].
Finger Millet [*Eleusine coracana* (L.)]	Rich in Fe, Zn, and lysine (17–65 mg/g of protein) compared to other millets. Total phenolic contents reported are 168 mg/100 g (pearl millet) and ferulic acid equivalents in the soluble phenolic fraction. Total flavonoid contents have been reported as 203–228 mg/100 g (finger millet), catechin equivalents in the soluble phenolic fraction[29].
Proso Millet [*Panicum miliaceum* (L.)]	Rich in Fe, Zn, and lysine (17–65 mg/g of protein) compared to other millets. Total phenolic contents reported are 168 mg/100 g (pearl millet) and ferulic acid equivalents in the soluble phenolic fraction. Total flavonoid contents have been reported as 140 mg/100 g (proso millet) catechin equivalents in the soluble phenolic fraction[10].
Barnyard Millet [*Echinochloa esculenta* (L.)]	Functional constituents, viz. g-amino γ-aminobutyric acid (GABA) and β-glucan, are used as anti-oxidants and in reducing blood lipid levels[32].
Kodo Millet [*Paspalum scrobiculatum* (L.)]	High magnesium content (1.1 g/kg dry matter)[24].

3. Nano-Fertilizer:

Fertilizers play a crucial role in the production of agricultural products, accounting for 35-40% of agricultural output. Some fertilizers have a direct impact on plant development. According to Ombedi and Saigusa[35], a significant amount of fertilizers applied in the environment is lost, with 40-70% of nitrogen (N), 80-90% of phosphorus (P), and 50-90% of potassium (K) not reaching plants. This inefficiency can lead to long-term financial losses for farmers.

To encourage the use of fertilizers, particularly urea, and to boost agricultural productivity, the government provides subsidies to reduce costs. However, unbalanced fertilization, nitrate contamination in the soil, and repeated application of fertilizers have negatively affected the natural balance of the soil. A promising solution to these issues is the development of fertilizers based on nanotechnology, which focuses on creating more effective fertilizer products. In addition to providing stress tolerance, the nano fertilizer triples the efficiency of nutrient usage (NUE).

The gradual and targeted efficient release will also take place since these nanofertilizers contain growth promoters, nutrients, and nanoscale polymers. When weighed against the prices and needs of chemical fertilizers, nano fertilizers are more cost-effective and may be used in lower quantities.

The U.S. Environmental Protection Agency defines nanomaterials (NMs) as substances containing particles with at least one dimension between 1 and 100 nanometers (nm). Nanofertilizers (NFs) are a specific type of NM that can function as carriers for traditional chemical fertilizers, enhancing nutrient efficiency and acting as sources of macro- or micronutrients for crop plants.

Thanks to their unique mechanisms of action, NFs are generally more effective than conventional fertilizers. They improve nutrient utilization, reduce nitrogen loss, and have a minimal environmental impact.

Nanotechnology employs nanoscale or nano-structured materials that have been researched as controlled-release vectors or fertilizer carriers. This has resulted in the creation of "intelligent fertilizers," which improve nutrient uptake and lower emissions[36]. Due to their small size and significantly increased surface area, NFs can be easily absorbed by plants, and their general mechanism of uptake by plants is shown in Figure 3.

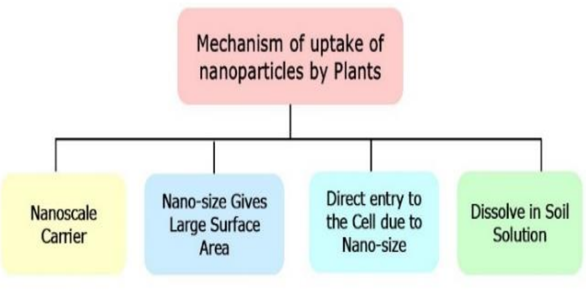

Figure 3. Generalized Mechanism of Nanoparticle Uptake

4. Agronomic Biofortification:

The agronomic approach to plant micronutrient biofortification aims to quickly and effectively address the deficiencies of essential elements in soil and plant life. This method focuses on producing a variety of vitamins and minerals. Fertilization is a key component of this technique, as it enhances the micronutrient content of crops such as legumes and grains. It's important to note that in developing countries, the agronomic biofortification strategy can be particularly beneficial[37]. To enhance micronutrient content in crops through agronomic biofortification, White and Broadley[38] recommended using phytoavailable micronutrient fertilizers, regularly correcting soil alkalinity, practicing crop rotation, and introducing beneficial soil microbes deliberately. Additionally, Graham et al.[39] discussed agricultural tools designed to improve crop nutrient levels, including crop systems, soil amendments, and fertilizers. Research indicates that micronutrient fertilization not only increases agricultural yields but also improves the nutritional quality of crops, thus addressing the related issues of human micronutrient deficiencies and health problems[30].

About one-third of the world's population consumes foods that are low in zinc[41]. Zinc is vital for the hormonal regulation of carbohydrate metabolism and serves as an essential micronutrient in various enzymes. The human body absorbs zinc primarily in the form of zinc gluconate. A deficiency of zinc in soil leads to low uptake of this mineral by plants. Two common sources of zinc are zinc sulfates ($ZnSO_4$) and zinc oxide (ZnO). ZnO nanoparticles, because of their high surface area to volume ratio and low volatility, are effectively absorbed, stored, and metabolized by plants to address zinc deficiencies[42]. Research has shown that applying ZnO nanoparticles at a rate of 5 mg per kg of soil increases the zinc concentration in sorghum (*Sorghum bicolor* (L.) Moench) and finger millet (*Eleusine coracana* (L.) Gaertn. ssp. coracana) grains by 94%. Additionally, seed priming with ZnO nanoparticles at a concentration of 5 ppm increases the zinc content of the grains by 13.96% compared to control plants[43]. Significantly, ZnO nanoparticles have not been associated with any unique toxicity or hazards at the nanoscale.

Foliar spraying of nano-chelated fertilizer in paddy fields is an effective method for enhancing rice biofortification. The application of nano chelated iron fertilizer significantly increases the nitrogen, phosphorus, and potassium content in white rice. Additionally, it promotes greater plant height, longer panicle length, increased grain weight, and higher paddy production compared to untreated controls. After using nano chelated iron fertilizer, rice crops exhibit elevated levels of protein and macronutrients. This suggests that the fertilizer enhances macronutrient absorption while reducing the reliance on chemical fertilizers. The most significant improvements in the quality and quantity of rice occurred during the nursery and booting phases when 2.5 g/L of nano chelated iron fertilizer was applied. This method proved to be cost-effective while requiring only a tiny amount of fertilizer. Iron in the soil can be present as maghemite (γ-Fe_2O_3), hematite

(α-Fe_2O_3), or magnetite (Fe_3O_4). However, due to various transformation processes in the soil, iron can become difficult for plants to absorb. Sufficient iron levels promote increased chlorophyll content, enhanced root development, and better seed germination. According to Kumar et al. (2021), priming finger millet (*E. coracana*) seeds with 100 ppm of Fe_3O_4 nanoparticles resulted in a 12.3% increase in the grain's iron content compared to treatment with $FeSO_4$.

5. Conclusion

Both plant and human health are greatly affected by deficiencies in micronutrients. One of the most effective strategies to ensure global food security is the development of nutrient-enriched crops through sustainable agricultural practices. This text outlines the most common methods of biofortification using nanomaterials. Nanotechnology-based techniques can contribute to the production of nutrient-rich foods by minimizing losses from soil leaching and volatilization. Additionally, these techniques can aid in genetic transformation processes that enhance the absorption, translocation, and accumulation of micronutrients. As a result, these methods can effectively biofortify food crops, helping to sustainably reduce human micronutrient deficiencies. When applied in appropriate quantities, micronutrient-enriched nanofertilizers pose minimal environmental risks while generally offering agronomic benefits, such as improved crop health and increased soil fertility. Agronomic biofortification can significantly enhance both yields and the nutritional quality of certain crop-micronutrient combinations, particularly those involving zinc and iron. Furthermore, nanoparticles, due to their smaller size and larger surface area, can replace conventional fertilizers. Numerous studies indicate that nanoparticles enhance the digestion and absorption of nutrients in grains, which, in turn, can help alleviate human nutritional deficiencies.

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Authors Contributions

Kushwaha P did the resources, writing the original draft of the paper and performing writing this review article. Verma R did the editing and correction of the paper. Kushwaha P and Verma R did the process of investigation and conceptualization. Singh A. K and Dixit P supervised the paper and had close supervision in the process of preparing the paper. All authors have read and agreed to the published version of the manuscript.

Conflicts of Interest

Authors declare no conflict of interest.

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