Sustainable Plant Nutrition in a Changing World

Vishnu D. Rajput · Hassan El-Ramady
Sudhir K. Upadhyay · Tatiana Minkina
Bilal Ahmed · Saglara Mandzhieva
Editors

Nano-Biofortification for Human and Environmental Health

Springer

Sustainable Plant Nutrition in a Changing World

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Preface

Nanotechnology has shown great potential in all spheres of life. Due to increasing pressure to meet food needs for increasing human population, novel agricultural innovations are required to enhance the health of edible crops and per unit area yield without impacting the associated environment in a negative way. In this regard, recent advancements in nanotechnology-based agricultural solutions have proven to be exploited to overcome the problems in agriculture that are associated with runoff of essential fertilizers from agricultural soils; low nutrient accumulation by crops; control of insects, pests, and seasonal biotic factors; treatment of wastewater used for irrigation; and plant uptake of xenobiotics (heavy metals, pesticides, industrial chemicals, drugs, etc.) present in contaminated soils. Due to these, the overall crop performance is compromised and crop yield is unwantedly reduced. Additionally, the consumption of such crop produces results in malnourishment and plant-mediated transfer of toxic substances among humans especially underprivileged and rural population. To date, the most convenient approach to tackle nutrient deficiency in plants is through the application of chemical fertilizers, but this method suffers from lower uptake efficiency of these added nutrients by plants. The other methods to combat nutrient deficiency are through fortification of nutrients by strategically adding supplements and diet modifications. These approaches have good results but unfortunately are not affordable to farmers and sustainable to the environment. To overcome such issues, nano-formulations being used as nano-fertilizers providing sustainable and prolonged localized release of specific nutrients and nano-pesticides to control the crop losses have tremendous potential and have been proven at pilot scales quite often.

Besides, nanotechnology-based formulations or tools can be applied to meet other difficulties in agriculture including enhancing crop yield, protection from insects and pests by use of nanopesticides, and early detection of contaminants by nano-biosensors to carry out remediation of polluted soils. Also, various types of nanomaterials including carbon nanotubes, metal, and metal-oxide nanoparticles have also been used as agents to stimulate plant growth. Another application of nanotechnology in agriculture is the enhancement of a particular nutrient or element in crop plants for human nourishment by either directly inducing its uptake or indirectly enhancing the intracellular levels of other associated elements that ultimately boost the synthesis of desired nutrient in plants. While considering the several advantages of nanomaterials for agriculture or human health, the competence and fate of nanomaterials in soil ecosystem should also be taken into account that is influenced by the ability of applied nano- material/particle/formulation to interact with soil constituents governed by the physicochemical properties of soil and nano-enabled product.

Nanomaterials can enter the environment through both anthropogenic and natural causes. Studies on the effects and use of nanoparticles in animals, plants, microorganisms, and soils have increased during the past ten years. Although these studies lack information on how nanoparticles affect biotic and abiotic factors, they are nonetheless crucial for the deployment of nano-fertilizers since they may have significant effects, either positive or negative, on ecosystem components. Hence, it was impossible to avoid this element of the application of nanotechnology in agriculture. It is necessary to recreate similar environmental conditions on a lab scale using a carefully chosen set of agriculturally relevant species in order to ensure the sustainable and secure usage of nanotechnological products in agriculture.

While considering all the pros and cons of applications of nanotechnology products in agriculture, we emphasize the judicial use of those nano-enabled products without compromising the sustainability of the environment and human health. Therefore, we have comprehensively drafted a list of chapters that will address major aspects related to nanotechnology, agriculture, and human health that will present thorough current knowledge for researchers of the field and academicians.

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Contents

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Vishnu D. Rajput Presently, Dr. Rajput is a Leading Researcher (Associate Professor), Head of Soil Health Lab at Southern Federal University, Russia. His ongoing research is based on soil contaminations, i.e., potentially toxic elements, and metallic nanoparticles, and investigating the bioaccumulation, bio-/geo-transformations, uptake, translocation, and toxic effects of metallic nanoparticles on plant physiology, morphology, anatomy, the ultrastructure of cellular and subcellular organelles, cytomorphometric modifications, and DNA damage. He comprehensively detailed the state of research in environmental science in regard to "how nanoparticles/heavy metals interact with plants, soil, microbial community, and the larger environment." He has published more than 400+ scientific publications cited 4813 times with 34 H-index. He is an internationally recognized reviewer (peer-reviewed 260 manuscripts for reputed international journals) and received an outstanding reviewing certificate from Elsevier and Springer. He is an editorial board member of various high-impact journals. He received "certificate for appreciation 2019" "Certificate of Honor 2020" Diploma Award 2021, 2022, from Southern Federal University, Russia, for outstanding contribution in academic, creative research, and publication activities.

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Hassan El-Ramady is Full Professor of Soil Fertility and Plant Nutrition at Kafrelsheikh University. His received his Ph.D. from Braunschweig University, Germany, in 2008. He is working mainly on the biological plant nutrition and how to overcome different problems in this field depending on the applications of nanotechnology particularly under stress. The area of scientific interest is plant nutrition and soil science, biogeochemistry of trace elements in soils, and environmental soil chemistry, soil nano-remediation, and nano-phytoremediation. He had several postdoctoral scholarships in Hungary, Germany, and Italy (during the period from 2012 to 2021) and has attended several international conferences in the USA (2012 and 2014), Austria (2013), Brazil (2015), and Germany (2004–2017). He is a member in many international societies from 2011 till now like American Society of Soil Science, German Society of Soil Science, and German Society of Plant Nutrition, and is a board member of Egyptian Soil Science Society. Currently, he is Editor-in-Chief, and Associate Editor of some journals like *Frontiers in Soil Science*, *Egyptian Journal of Soil Science* (EJSS), and *Environment, Biodiversity and Soil Security* (EBSS), besides being a guest editor of some journals like *Plants* (Mdpi). He has peer reviewed more than 162 research or review articles for various international publication media, and he is editor of about 200 articles and has several book chapters (30), more than 102 articles, 176 published materials. He was the lead editor of book *The Soils of Egypt*, which was published by Springer in 2019.

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Chapter 1
Nano-Enabled Approaches for Biofortification Strategies to Enhance Crop Production with Micronutrient Enrichment

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Contents

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1.1 Introduction

Nanotechnology's potential in agriculture is being pursued because of the increasing food requirement to sustain the fast growing population throughout the globe. Studies have predicted that by the year 2050, the world's population will have reached around 9.6 billion (Bongaarts, 2009). Human activity and societal shifts are reducing the productivity of farmlands (Tarafdar, 2011). Famine and hunger are possible results, so it's imperative that we work together to find ways to make plants more productive (Tarafdar, 2011). Precision agriculture, which uses nanotechnology as cutting edge of technology, is developing strategies to keep up with the ever-increasing demand for food (Rajput et al., 2021). Technologies which have potential to uplift nutrient-rich agricultural productivity, are budget friendly, have high resource use efficiency, guarantee nutrient security, enhance the production value, boost farmers' economies, and deliver an agri-value chain to rural participants without posing any harm to the natural environment are being adopted away from the conventional methods of crop production (Pimentel, 1991; Xiong et al., 2017). This innovation takes advantage of the nanoscale properties of enhanced materials to increase the value of agricultural production. In agriculture, nanotechnology is filling in the gaps caused by nutrient leaching and crop fortification (Thiruvengadam et al., 2018). In the nano-regime, farmers are employing this technology to increase both the quality and yield of their harvests. Nanotechnology has many potential uses in agriculture, such as nanobiotechnology, livestock, nanotoxicology, agrochemicals, hydroponics, biotechnology, etc. (Thiruvengadam et al., 2018).

In an effort to increase the likelihood that plants will absorb the added nutrients, fertilizers are fortified with plant-necessary nutrients (Kabiri et al., 2017). Plant show the nutrient deficiency symptoms through many ways including unusual growth of the plant parts; however, in some cases, even the plants grown in micronutrient-rich soil may show deficiency symptoms due to inability of roots to uptake and transport the nutrients because of their small pore size (Rajput et al., 2021). Therefore, it is crucial to investigate ways of improving the nutrient status of plants, thus ensuring crop quality in order to fulfill the rising population's demand for food.

Chemical fertilizer use has greatly increased crop yields and has been a long-standing practice (Kabiri et al., 2017). However, they cause an imbalance of soil minerals, disturb the soil structure, nutrient status of soil, and overall ecosystem, all of which together have long-term devastating effects (Dietz & Herth, 2011). It is important to create intelligent substances that can liberate nutrients to particular areas as and when required and support a pollution-free environment in order to deal with the situation. According to the recent research, grapheme has come out as an effective material that can carry plant nutrients (Kabiri et al., 2017). It can increase crop production by releasing nutrients to the plants in a slow, controlled manner and also has little negative impact on the environment (Kabiri et al., 2017). As the global nanotechnology market reached $1 trillion in 2015, it appears that nanotechnology has the potential to complement improved agriculture (Roco, 2011).

The majority of people's health depends on the quality of the food they eat, and plants continue to be our primary source of nourishment (Yang et al., 2007). Foods that become staples in a community's diet are typically those that are high in calories and are consumed on a regular basis in large quantities (Pimentel, 1991; Verma et al., 2022). Therefore, staple foods and nutrition status of its consumers hold a strong relationship, particularly among people living in rural and poor areas, where supplementary nutrients are scarce. Foods often lack necessary micronutrients in these regions. They have serious consequences and have spread around the world (Hossain & Mohiuddin, 2012). Half of all kids go without the nutrition they need, making them more susceptible to illness and reducing their cognitive potential. Iron and zinc deficiencies are responsible for a significant amount of infant mortality and worldwide health issues (Clemens, 2014). Dietary diversification, drug therapy, and industrial fortification are just a few of the proposed approaches to resolving nutrient deficiencies (Datta & Vitolins, 2016). Food diversity is recommended as a long-term solution, but it is out of reach for the poor, who are already at risk of malnutrition. With the exception of iodized salt, industrial fortification of food nutrients has not been particularly effective. Biofortification is the practice of boosting the nutritional value of crops before, during, and after harvest (Bouis et al., 2011). Biofortification is different from other interventions to address micronutrient deficiencies because it is cheap and accessible to all people (Bouis, 2018). This is due to the fact that many people rely on these fortified crops as a staple in their diet. Because of this, it has no additional expenses and is unaffected by people's social habits (Datta & Vitolins, 2016). Vegetables and other non-staple foods are expensive despite their high vitamin and mineral content (Bouis et al., 2011). Many low-income people put the majority of their food budget toward staples that provide only minimal amounts of energy, leaving them with little to no money for more expensive items like fruits, vegetables, and meat (Stein et al., 2007).

Agronomic and breeding techniques are among the biofortification strategies that are frequently utilized for enhancing nutritional status of crops (Stein et al., 2007). Fertilizers, both inorganic and organic, and biofertilizers are highlighted as part of agronomic interventions. Organic matter is difficult to use because of its significantly less mineral content and prolonged nutrient release time, while inorganic fertilizers, which are generally available in sizes greater than 100 nm, are prone to volatilization and leaching. Many efforts to biofortify crops by enhancing their ability to absorb nutrients have met with limited success. As a result, now is a great time to employ nanotechnology to address some of these issues.

1.2 Nanoparticles Synthesis

Top-down and bottom-up approaches are two strikingly common classical methods for fabricating NPs of the desired shape and size, respectively (Gwo et al., 2016). Both methods, despite their differences in synthesis principles, generate NPs with the desired properties.

1.2.1 Top-Down Approach

The top-down strategy involved shattering large materials into smaller and smaller pieces until they were finally reduced to NPs of the finest possible size. The manufacturing processes for these NPs included photolithographic methods, grinding, sputtering, and milling (Meyers et al., 2006). All the techniques possess advantages and disadvantages in terms of NP production efficiency. The top-down method is a practical strategy that can easily generate a substantial number of NPs. The problems with top-down manufacturing include imperfect NP surfaces and the possibility of NPs being damaged during production (Thakkar et al., 2010). Top-down synthesis of NPs is limited in some cases because the optical and physiochemical properties of the NPs depend on the structural design of NPs' surface.

1.2.2 Bottom-Up Approach

The bottom-up method consists of separate assembly of atoms, molecules, or clusters to produce a wide variety of NPs is also popular (Decarolis et al., 2020). Many different methods are used to construct NPs which also include self-assembly of monomer/polymer molecules, chemical or electrochemical nanostructural precipitation, sol-gel processing, laser pyrolysis, chemical vapor deposition (CVD), plasma or flame spraying synthesis, and bio-assisted synthesis (Dhand et al., 2015). So, bottom-up is a flexible method for making nanoclusters with a wide range of potential uses.

1.3 Methodologies for Synthesis of Nanoparticles

1.3.1 Physical Methods

Physical techniques (Fig. 1.1) such as deposition, sputtering, ball milling, and plasma-based methods are used to construct nanomaterials (Dhand et al., 2015). Most of these techniques produce metal nanoparticles at a very slow rate. Ball milling techniques, for instance, have a yield of nanomaterials of 50% or less (Yadav et al., 2012). Sputtering technique produces very less amount (6–8%) of sputtered material of size less than 100 nm which can generate a large particle size distribution. For laser ablation and plasma techniques, a high-energy consumption is necessary. Most of the physical methods are very expensive and cannot be used for real-world commercial applications due to their irregular size distribution, slow rate of production, nonrecyclable byproducts, and immense energy consumption (Seetharaman et al., 2018).

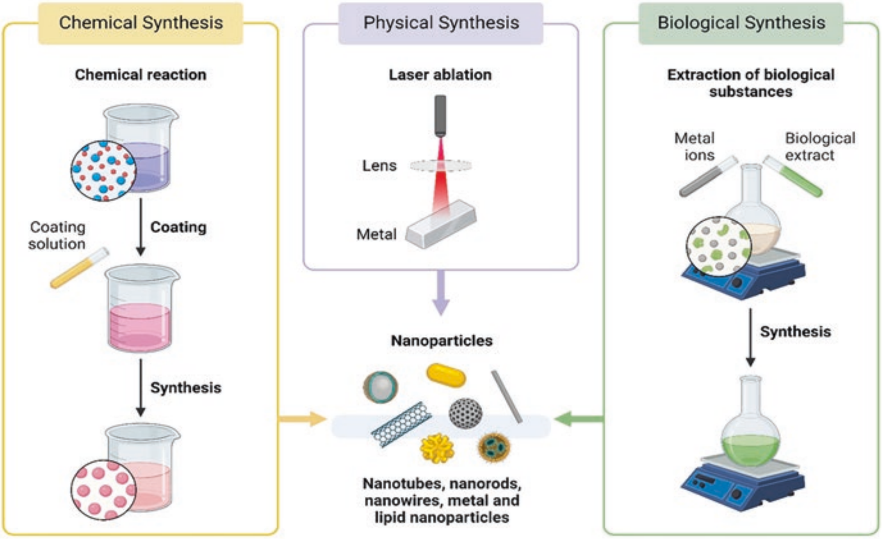

Fig. 1.1 Different methods, like physical, chemical, and biological methods, are used for the synthesis of nanoparticle that is further used for nano-enabled approaches for biofortification

1.3.2 Chemical Method

Numerous chemical processes have been proposed for the nanoparticles creation, and most of these processes are frequently exploited to produce unstructured materials (e.g., chemical reduction, pyrolysis, sol-gel method, microemulsion, polyol synthesis, hydrothermal synthesis, chemical vapor deposition) (Darroudi et al., 2013). Chemical method require hazardous chemicals for nanoparticle synthesis and also produce harmful substances as by-product which can pose serious problems to human health and the ecosystem (Zhang et al., 2019). Therefore, the use of such NPs specifically in biological applications is restricted.

1.3.3 Biogenic Method

Green synthesis, also known as biosynthesis or biogenic methods, is a nonhazardous, low-cost, and time-efficient method of producing NPs. Biological systems such as bacteria, fungi, viruses, yeast, actinomycetes, plant metabolites etc. can be used for metal and metal oxide NPs. Biogenic methods for the synthesis of NPs are classified into the following two main categories:

1.3.4 Plant Extract-Based Biogenic Synthesis of NPs

The utilization of plant extracts or biomass for the biosynthesis of NPs is a rapid, clean, harmless, and environmental-safe option (Iravani, 2011). NPs developed from noble metals, metal oxides, bimetallic alloys, etc. are generally synthesized using this technique (Mittal et al., 2013). The rate of photosynthesis of NPs is comparable to or even exceeds that of chemical routes, allowing it the most efficient biosynthesis method (Akhtar et al., 2013). Lemongrass leaf extract treated with aqueous AuCl ions was used as biogenic NPs (Chandran et al., 2006). It has also been reported that *Tamarindus indica*, aloe vera, *Emblica officinalis*, etc. leaf extracts can be helpful when constructing Au-NPs. Pd-NPs and Pt-NPs with sizes on the nanometer scale were synthesized from plant extracts (Ankamwar et al., 2007; Chandran et al., 2006). Extracts of *Azadirachta indica* leaves and *Emblica officinalis* fruits are converted into highly concentrated Ag NPs. Plant materials such as aloe vera, *Capsicum annuum*, and *Helianthus annuus* leaves to produce silver nanoparticles (Ag NPs) with high yields and purity (Chandran et al., 2006). Peptides and terpenoids which are used to stabilize Cu-NPs are found in the stem latex of the medicinal plant *Euphorbia nivulia*, which were used in their extracellular production (Li et al., 2007). A very intriguing study describes the biosynthesis of In_2O_3 NPs (5–50 nm) using aloe vera plant extract (Serda et al., 2008). Furthermore, *Sedum alfredii*, a Zn-hyper-accumulator plant, yields ZnO NPs with an average size of 53.7 nm (Qu et al., 2011). Biomass from the *Medicago sativa* (alfalfa) plant was used in a biogenic synthesis of iron-oxide NPs (Herrera-Becerra et al., 2008). In situ fabrication of shape-selective silver nanostructures uses curcumin as a stabilizing and reducing agent. Glutathione, an antioxidant tripeptide, is found in plants, animals, fungi, and archaea.

1.3.5 Microbe-Based Biogenic Synthesis of NPs

Many different types of microorganisms, including bacteria, actinomycetes, fungi, algae, and yeast, can be exploited as bio-agents during the NP development. Extensive scientific efforts were put forward for the development of this method of production, which produces a broad range of NPs (Ag, Au, Pd, TiO_2, CdS, etc.) (Zhang et al., 2011). Microorganisms are able to extract metals from their environments by snatching up target ions and using enzymes produced by cellular activities to convert the ions into metal. Based on where NPs are synthesized, this process can be categorized as either intracellular or extracellular. Intracellular delivery of metal ions allows NPs to be formed inside the microbial cell with the help of enzymes. It is necessary to entrap metal ions over cell surface and reduce ions in the presence of enzymes during the extracellular synthesis of NPs. Bacterial biomass contains a variety of anionic functional groups that the bacteria use to reduce interacting metal ions. *S. aureus* bacteria can be used for extracellular synthesis of antimicrobial Ag

NPs. The biomatrix of the bacteria was used in the nonenzymatic development of Ag NPs, as both reducing and capping agent (Sintubin et al., 2009). For the biosorption and subsequent reduction of silver cations, Gram-positive bacteria, for the biosorption and further reduction of silver cations, require the presence of an anionic-functional-group-rich cell wall composed of peptidoglycan, teichoic acids, lipoteichoic acids, proteins, and polysaccharides. The biofactories for the production of TiO_2 NPs in the 40–60-nm size range were based on the lactobacillus bacterium (Prasad et al., 2007). A new hyper metal-resistant *Bacillus cereus* PGNI bacterial strain that forms intracellular crystalline Ag NPs was recently isolated (Ganesh Babu & Gunasekaran, 2009). Semiconducting CdS nanocrystals were also synthesized using a bacterial system (wurtzite crystal structure). In yet another intriguing study, researchers looked into the feasibility of using marine bacteria as a source of AuNPs.

1.4 Role of Nanotechnology in Crop Fortification

New studies suggest that nanotechnology may 1 day completely alter our agricultural methods (Dimkpa & Bindraban, 2016). This opens up the possibility of using agrochemicals with a sophisticated delivery structure that is risk-free, target-specific, and simple to implement. Compared to even the most cutting-edge polymeric-type conventional fertilizers, nanofertilizers provide greater efficiency because of their elevated surface area-to-volume ratio. Because of their composition, they may also be able to be released gradually, facilitating the plants' efficient uptake of nutrients. Therefore, this technology provides a foundation for developing novel and sustainable nutrient delivery systems that take the benefit of the nanoporous surfaces of plant parts (Fig. 1.2b, c). Encapsulated nanoparticles, nanoclays, and zeolites improve fertilizer efficiency, reinstate soil fertility (Fig. 1.2a), and plant health without causing environmental pollution and agroecological disturbance (Manjunatha et al., 2016).

Zinc oxide nanoparticles (ZnONPs), silica, iron, and titanium dioxide, ZnS/ZnCdSe core-shell quantum dots (QDs), InP/ZnS core-shell QDs, Mn/ZnSe QDs, gold nanorods, Al_2O_3, TiO_2, CeO_2, and FeO are all potential ingredients in nanofertilizers (Prasad et al., 2017). However, the size, concentration, composition, and chemical properties of nanomaterials, among other factors, all play a role in whether or not using nanomaterials as fertilizers is successful for a given plant species (Thakur et al., 2018). Thus, it is necessary to further study the area of nanotechnology-based agriculture for sustainable crop production. This can only be achieved with the help of new technologies, which in turn require a deep understanding of biology, biotechnology, material science, and engineering.

The nutrients carried by traditional fertilizers are prone to leaching loss and contaminate underground water aquifers. The search for alternatives, such as the nanofertilizers, is in response to the serious environmental problems caused by chemical fertilizers, such as greenhouse gas emissions and hypoxia (Dimkpa &

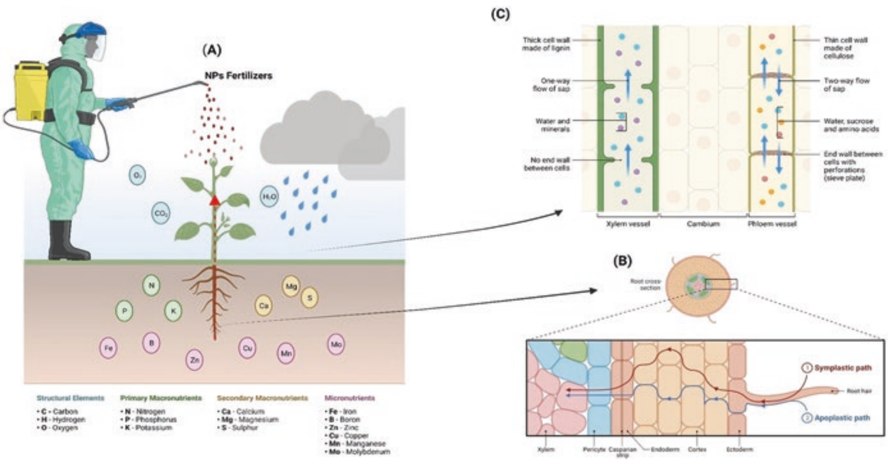

Fig. 1.2 Diagrammatic representation of mechanism of action of nanoparticle-based fertilizer in the soil (**a**) and plant system (**b** and **c**) that transport via symplastic and apoplastic pathways through the xylem and phloem in different parts of the plants

Bindraban, 2018). In addition to its many other benefits, nanofertilizer has the unique property of allowing nutrients to be liberated at slow pace, thus reducing the likelihood of nutrients being leached away.

Because of their small size, high surface-to-volume ratio, and exceptional optical properties, nanomaterials are in a class all their own. Nanofertilizers are advantageous due to their properties, which allow for advancements in plant growth, nutrient security, and a wide range of farming methods. With increasing number of researches and development in the area of nanotechnology applications, many different industries have begun using this cutting-edge science to develop cutting-edge goods. The human population is expanding, so it stands to reason that agricultural output would also benefit from expansion. Fertilizers have been useful in increasing food production, and with the help of nanotechnology, new and better fertilizer varieties, called nanofertilizers, can be manufactured. They are readily taken up by the ground, improving soil quality and consequently plant growth. Although nanotechnology has many potential applications, the majority of research and development efforts have been focused on electronics, optical devices, water purification, and healthcare. Because of the vigorous nature of nano-size materials and the fact that conventional fertilizers lack all the elements necessary for crop development and nutritional composition, there is a great scope of developing materials that can carry nanofertilizers which in turn can be utilized as the eco-friendly solution of nutrient related problems (Dimkpa & Bindraban, 2018).

Nanofertilizers have many advantages over traditional fertilizers, including the ability to increase crop resilience to drought and disease while simultaneously reducing chemical fertilizer use by three times. Because of their high surface area-to-volume ratio, they are readily absorbed by plants. But the degree to which plants can absorb nanoparticles from the soil is strongly influenced by the particles' sizes and shapes. It's possible that the nanoparticles need to undergo a chain reaction, including oxidation and recombination before they can be absorbed by plants and used as a source of the necessary micronutrients. The nanosized characteristic of the nutrients makes fortifying the plant with them an intriguing prospect. The plant's ability to multiply storing such nutrients fills the gap caused by the lack of those nutrients. In addition, specific nutrient deficiencies in plants could be corrected through the use of nanofertilizers that have been engineered for this purpose. Different arrangements of the atoms on the nanomaterial surface can be used in a variety of ways to produce the desired effects.

1.4.1 Zeolite-NP-Based Nanofertilizer

For effective crop production and lessening of environmental vulnerabilities related to agricultural activities, zeolites, which are mineral materials, are used because they protect the soil by conserving soil nutrients available in soil (Morales-Díaz et al., 2017). Over 50 different mineral types of basic alkali and alkaline earth aluminosilicates make up the natural zeolite that has recently been developed as a nanofertilizer. This is because it has been used in the production of maize due to its accessibility and low cost (Eroglu et al., 2017). It is not only a smart carrier and regulator of chief mineral fertilizers but it also provides few minerals to the plants themselves. As a carrier for nitrogen and potassium, zeolite allows for less fertilizer to be used for the same effect (Reháková et al., 2004). It was discovered that when zeolite compound was combined with humus materials, crop yields increased. It has been reported that zeolite nanocomposites containing nitrogen, phosphorus, and potassium (NPK), as well as other nutrients and micronutrients such as amino acids, mannose, Ca, Fe, and Zn, have helped crops grow and are absorbed by those crops. Because of zeolite's limited capacity to take in and store anions, biopolymers are added to improve its performance in this regard (Morales-Díaz et al., 2017).

1.4.2 Zinc/Zinc Oxide NP-Based Nanofertilizer

Zinc oxides (ZnO) and zinc sulphates are the most common forms of zinc used in fertilizer fortification. It would be impossible to overstate the importance of zinc to living organisms, as it is a component of most enzymes, is required for hormone and chlorophyll regulation, and has a key importance in carbohydrate metabolism. ZnO is easily absorbed, metabolized, and accumulated in plant systems when it is in the form of nanoparticles. Zinc is a micronutrient, so only small amounts are required; however, excessive ZnO NP concentrations can stunt plant growth and cause abnormalities in germination, root development, and early-stage seedling biomass (Singh et al., 2013). However, plants' growth and development may be stunted if they don't get enough of it.

Zinc deficiency in the soil causes its inadequate absorption by the plants, which in turn leads to nutritional deficiencies in both humans and animals. As per the report generated by the World Health Organization, one-third of the population of the world is suffering from zinc deficiency because of low zinc content especially in staple crops (Mortvedt & Giordano, 1967; Sadeghzadeh, 2013). Diseases of "hidden hunger" can be caused by a lack of zinc and other micronutrients in the food we eat. The body has a daily need for this micronutrient that, when not met for an extended period of time, leads to serious illness because the body cannot produce it. By fertilizing crops with sufficient micronutrients, this could be avoided.

Because ZnO is insoluble in water, it is combined with zinc sulphate to create a fertilizer. In the alkaline soils, crops are more prone to zinc deficiency which ultimately reduces crop productivity (Sadeghzadeh, 2013).

There is a need for a large quantity of these micronutrients, making it challenging to apply them to the soil in a uniform manner. Unlike zinc, zinc oxide and zinc sulphate are only moderately effective when used alone as micronutrients (Mortvedt & Giordano, 1967). These are efficiently utilized long with micronutrients, which act as carriers. Since 2.0% of zinc could be dissolved in polyphosphate, ammonia, and anhydrous ammonium nitrate (AAN), but only 0.05% of zinc is absorbed in orthophosphate, the carriers also play a role in the dissolution and absorption of the micronutrients, especially when they are liquids (Mortvedt & Giordano, 1967). The mineral makeup, ionic strength, and organic matter of the soil all play critical roles in nutrient uptake and bioavailability. Solubility and absorption of zinc are affected by the presence of calcareous soils, which have an elevated pH and calcium carbonate ($CaCO_3$) concentration (Milani et al., 2015). Zinc carbonate forms as a precipitate when calcium carbonate is present, and Zn is exchanged for calcium. Physical application of micronutrients to the soil has found to have nonsignificant effect on crop nutrient status. Soil composition can also be a problem for plants trying to take in micronutrients; sandy soils, for example, don't do a very good job of holding on to the fertilizer you put on them because it washes right through. The availability of soil micronutrients is also influenced by the soil's pH level. With the exception of molybdenum, the availability of micronutrients decreases as soil pH rises. As the pH of soil increases, zinc gets precipitated into $Zn(OH)_2$, $ZnCO_3$, and Zn_2SiO_4 and is adsorbed on the soil surface alongside other clay and inorganic materials as the soil pH rises (Milani et al., 2015). Such actions reduce the system's solubility, which prevents soil minerals from being efficiently desorption by plants.

However, nanozinc oxide has vast agricultural applications, including but not limited to genetic manipulation of crops; nanofoods; recommended diets for some sick people; soil recovery of lost nutrients; act as blocking agent for against excess UV radiation during plant growth; and application as fertilizers in crop production.

1.5 Conclusion

Nanotechnology continues to be at the forefront as a potential game-changer in the agricultural sector, despite reports about the dangers of some nanoparticles. It may offer even more benefits than nuclear power. These benefits include low toxicity of nanoparticles in comparison to other compounds, enhanced carrier system use efficiency, improved bioavailability, simple development and processibility and their manipulative ability that enhances the physicochemical properties. Synthesis methods that involve plant-based raw resources, waste vegetables, plant extracts, flowers, plant barks, roots, fruit peels, and leather cuttings can be deliberately utilized to make this field of science more environmentally friendly and, thus, can have sound application in agriculture. Nanoproducts need to be regulated to ensure the safety of the public, the people who use them, and the environment. Companies producing nanomaterials should be required to disclose this information to the public. It is time to move nanomaterials from the lab into practical use, despite the potential for a number of issues. To minimize potential risks, nanomaterials should be introduced and monitored during the early stages of crop production. They should be used for biosensing and to replenish soil nutrients during land preparation. In particular, these methods have the potential to enhance sanitary conditions, food security, environmental protection, and quality of life in developing nations.

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Chapter 2
Nanobiofortification: An Emerging Approach

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Contents

2.1 Introduction

It is well-recognized that the connection between mankind and several environmental factors is mediated by a complex web of relationships. Globally, changing climate is one of the most pressing environmental issues. In 2020, planet Earth was threatened by flooding, droughts and extreme snowstorms, preternatural locust attacks in Africa, and a worldwide corona virus disease outbreak. These challenges

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have devastatingly affected food security, the environment, and human health (Gibson & Hotz, 2001). Nutrition is essential for human health, and a lack of these nutrients leads to malnutrition, which is defined as a shortage of minerals and vitamins, among other things (Jiang et al., 2021). During the recent years, number of approaches have been developed to combat malnutrition, primarily through modern biotechnology, conventional breeding, and agronomic biofortification, which have significantly impacted sustainability. As reported by Thakur et al. (2018), "malnutrition affects at least one-third of the world's population, and its elimination remains a formidable challenge."

As the human population continues to increase, the need for more food necessitates using nanotechnology in agriculture. According to a population survey, it is anticipated that by 2050, there will be 9.6 billion people on Earth (El-Ramady et al., 2021). The latest technology for precision agriculture is nanotechnology, designed to address the demands of growing population regarding food production. Using nanoscale properties, this technology adds value to agriculture. The application of nanotechnology in agriculture is closing the gap in nutrient loss and fortifying crops. Using nanotechnology, farmers are using this science to enhance the quality and quantity of agricultural products. Nanotechnology can be applied to agriculture in many ways, including nanotoxicology, nanobiotechnology, agrochemicals, hydroponics, livestock, and biotechnology.

In order to achieve food security and healthy nutrition, nanotechnology can be used in urban agriculture. Nanomaterials in agriculture are intended to reduce nutrient losses, pests, and hazards while improving crop yields. Directly or indirectly human health is affected by all the elements of the environment (e.g., air, soil, drinking water, and edible plants), with agroecosystem microbes being a dominant component (Safarieskandari, 2019). Biofortification is the process of fertilizing edible plants with nutrients, which is essential to human health. In addition to wheat, rice, maize, cassava, and sweet potatoes, biofortified food crops include horticultural crops such as pear and strawberry. Several nutrients can be used in biofortification, including iron, iodine, boron, copper, calcium, zinc, and selenium. Vitamins can also be biofortified in edible crops in addition to nutrients, such as thiamine (vitamin B1), riboflavin (B2), niacin (B3), pantothenic acid (B5), pyridoxine (B6), biotin (B7), folates and their derivatives (B9), or ascorbate (vitamin C) or tocopherol (vitamin E).

2.2 Role of Nanotechnology in Biofortification

Advances in science and technology benefit the agricultural sector by offering new and better ideas and solutions to the world's most pressing issues. Nanotechnology has made it possible to manufacture more efficient and pollutant-free nanoformulations for agriculture sustainability and human health. After entering the complicated plant-soil system, these compounds have the potential to affect plants' physiology, and their effects can be studied to learn more about them. Nanotechnology is bridging gaps between nutrient loss and crop fortification and is used by farmers to increase the yield and quality of their crops. Many people regularly consume these fortified crops as stapled foods. Nanobiofortification is a novel strategy to enrich crops with critical nutrients to augment human diets with balanced diets containing nutrients to prevent malnutrition. According to Gibson and Hotz (2001), dietary modification or alteration can increase the micronutrient content and bioavailability of staples and other food sources. This innovative method has various advantages such as nano-fertilizers or nutrient-based nanoparticles.

It is possible to synthesize nanoparticles (NPs) in a manner that is less harmful to the environment by using plant extracts (seeds, leaves, flowers, and roots), microbes (bacteria, algae, fungi, and yeast), and biomolecules (enzymes, carbohydrates, and proteins), which represent biological substrates. Compared to physical and chemical approaches, the biogenic creation of nanoparticles is "a boon" to human health, more practical, affordable, and eco-friendly. The modified nanoparticles could be directly used as food additives or in the food business, such as colorants, emulsifiers, taste enhancers, sugar substitutes, foaming, and anti-foaming agents (Medina-Reyes et al., 2020). Different nanoparticles have been used as nanopesticides or nano-fertilizers, which can improve stressed cultivated plants by various mechanisms, including boosting the antioxidant defense mechanism, stimulating photosynthesis, and increasing water, nutrients, and phytohormones. Green-synthesized NPs have been used for various human health applications, including antimicrobial agents, zinc-NPs and silver NPs (Munir et al., 2020), and zinc oxide NPs (Umavathi et al., 2021).

2.3 Biofortification Approaches

Biofortification is an agricultural approach that aims to increase the micronutrient content of food crops such as rice, maize, wheat, and pearl millet. Increasing micronutrient intakes is possible through a number of approaches. These include genetic, agronomic, and nanotechnology biofortification of edible crops (Fig. 2.1). Agronomic biofortification can be accomplished by improving soil micronutrient phytoavailability or using micronutrient fertilizers. In genetic biofortification, the production of crop cultivars that have more uptake efficiency of micronutrients from the soil and incorporates them in edible quantities through genetic engineering or traditional breeding. These biofortification strategies aim to maximize the daily consumption of particular food items.

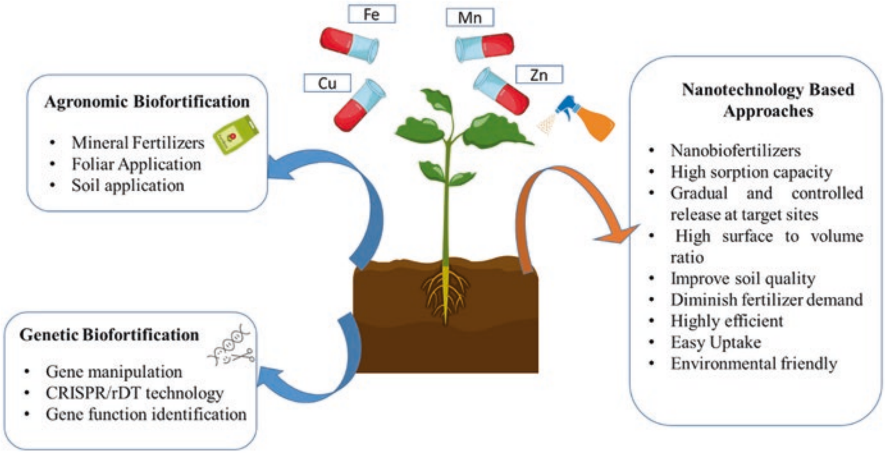

Fig. 2.1 Biofortication approaches

2.4 Agronomic Biofortification

Applying micronutrient fertilizer is a practical and speedy way to grow biofortified food crops that can be easily implemented in developing nations. Seed treatment and foliar fertilizer spray are the two methods for achieving biofortification. Micronutrients can also be used in combination with soil amendments to improve crop production and nutritional quality. Many researchers explored the effect of fertilizer combined with organic matter or manure and foliar application of micronutrients (Athar et al., 2020).

2.5 Genetic Biofortification

The transgenic approach is a promising way to make biofortified crops because it directly adds new genetic information to the plant genome. This technique is one of the most efficient and feasible option for genetically fortifying a crop with a specific micronutrient when that nutrient is not naturally present in that crop. Gene functions are identified and defined before being used to modify the plant's metabolism to diminish anti-nutrient factors while increasing the concentration of promoter chemicals and making them more bioavailable by increasing the concentration of micronutrients (Christou & Twyman, 2004).

2.6 Biofortification Through Nanotechnology-Based Approaches

One of the most significant issues that agronomic biofortification offers to the environment is the increased concentration of the compounds that are toxic such as nitrate, heavy metals, etc. in soil and water as chemical fertilizers are applied in greater quantities. Uncontrolled release of nutrients from chemical fertilizers decreases the crop grain quality. Many of these problems of agronomic biofortification could be addressed by nanotechnology. Reducing the particle size of applied fertilizers, plant roots can absorb more nutrients because there are more reaction sites, and nanosize particles are in a perpetual state of motion known as "the Brownian motion" (Jiang et al., 2018). Several properties of nanoparticles make them suitable for the production of nanofertilizers, including high sorption capacity and specific surface area and gradual and controlled release at target sites. Encapsulation of nutrient with nanomaterials results in effective absorption of nutrient by plants due to the controlled or gradual release of nanoparticles and simple entry of nanoparticles into the vascular system through biological barriers. Compared to conventional fertilizers, this constant long-term delivery of plants using nanofertilizers leads to higher crop production (Feregrino-Perez et al., 2018). Because of these advantages of nanofertilizers over chemical fertilizers, nanofertilizers are now consistently selected for micronutrient biofortification (Table 2.1).

Table 2.1 Impact of various nanoparticles for biofortification in different crops

Crop	Nanoparticle type	Concentration	Application method	Effect	References
Wheat (*Triticum aestivum* L.)	ZnO	50–1000 mg L^{-1}	Soil	Increase Zn content in grain	Du et al. (2019)
Wheat (*Triticum aestivum* L.)	ZnO	25–100 mg L^{-1}	Seed priming	Increased the grain Zn content	Rizwan et al. (2019)
Wheat (*Triticum aestivum* L.)	ZnO	1.7 mg kg^{-1}	Soil	Increase grain zinc concentration by 29%	Dimkpa et al. (2020)
Wheat (*Triticum aestivum* L.)	ZnO	75 and 750 mg L^{-1}	Foliar	Enhanced grain Zn concentration	Doolette et al. (2020)
Wheat (*Triticum aestivum* L.)	ZnO	0.96 kg ha^{-1}	Foliar	Enhanced zinc acquisition in seed	Tong et al. (2020)
Finger millet (*Eleusine coracana* L.)	ZnO	5 mg L^{-1}	Seed priming	Increased grain Zn content by 13.96%	Chandra et al. (2021)
Rice (*Oryza sativa* L.)	ZnO	20 mg L^{-1}	Seed priming	Zinc concentration raised from 21.08 to 33.14 ug g^{-1}	Sharma et al. (2021)

(continued)

Table 2.1 (continued)

Crop	Nanoparticle type	Concentration	Application method	Effect	References
Wheat (*Triticum aestivum* L.)	ZnO	40–120 mg L^{-1}	Foliar	Enhanced absorption of zinc	Sheoran et al. (2021)
Rice (*Oryza sativa* L.)	ZnO	25 and 100 mg kg^{-1}	Soil	About 13.5–39.4%incrase in grain zinc content	Yang et al. (2021)
Rice (*Oryza sativa* L.)	ZnO	25 mg L^{-1}	Foliar	Increased grain zinc grain by 55%	Parashar et al. (2023)
Wheat (*Triticumaestivum* L.)	ZnO	120 mg L^{-1}	Seed priming	Improved grain Zinc concentration (25.32 mg kg $^{-1}$ FW) in var. Zincole-16	Abbas et al. (2023)
Wheat (*Triticum aestivum* L.)	ZnO	100 mg kg^{-1}	Soil	Improved grain Zn content by 24%	Chen et al. (2023)
Wheat (*Triticum aestivum* L.)	ZnO	30 mg kg^{-1}	Soil	Improved grain Zn content from 31.7 to 49.0 mg kg^{-1}	Yadav et al. (2023)
Wheat (*Triticum aestivum* L.)	Fe	5–20 mg L^{-1}	Seed priming	Fe concentration in grains increased by 20–121%	Rizwan et al. (2019)
Wheat (*Triticum aestivum* L.)	Fe_2O_3	50 and 500 mg kg^{-1}	Soil	Enhanced Fe content	Wang et al. (2019)
Wheat (*Triticum aestivum* L.)	Fe_2O_3	500 mg L^{-1}	Hydroponics	Enhanced Fe uptake	Al-Amri et al. (2020)
Finger millet (*Eleusine coracana* (L.) Gaertn. Ssp. *coracana*)	Fe_3O_4	100 mg L^{-1}	Seed priming	Increased grain Fe content by 12.26%	Chandra et al. (2021)
Wheat (*Triticum aestivum* L.)	FeO	25–100 mg kg^{-1}	Soil	Increased iron uptake	Manzoor et al., (2021)
Sugarcane (*Saccharum officinarum* L.)	Cu	20–60 mg L^{-1}	Soil	73% higher Fe content	Tamez et al. (2019)
Wheat (*Triticum aestivum* L.)	CuO	50 and 500 mg kg^{-1}	Soil	About 18.84–30.45% higher cu content in grain	Wang et al. (2019)
Soybean (*Glycine max* (L.) Merr.)	CuO	50–500 mg kg^{-1}	Soil	Copper grain concentration improved by 1.8 times	Yusefi-Tanha et al. (2020)
Rice (*Oryza sativa* L.)	CuO	75 mg kg^{-1}	Soil	Enhance cu grain content	Deng et al. (2022)

(continued)

Table 2.1 (continued)

Crop	Nanoparticle type	Concentration	Application method	Effect	References
Barley (*Hordeum vulgare* L.)	Cu	500 mg L^{-1}	Foliar	Twofold increase in cu grain content	Jośko et al. (2023)
Rice(*Oryza sativa* L.)	Se	0.7896 and 2.3688 g L^{-1}	Hydroponics	Se concentration raised to 6.7-and 20.4-fold	Wang et al. (2020)
Coffee (*Coffea arabica* L.)	Se	10–160 mg L^{-1}	Foliar	Se content in coffee grains ranged from 4.84 to 5.82 mg kg^{-1}	de Brito Mateus et al. (2021)
Rice (*Oryza sativa* L.)	Se	1.974–7.896 g L^{-1}	Foliar	Se enhanced by 218.9–1096.6 μg kg^{-1}	Wang et al. (2021)

2.7 Nanobiofortication for Environmental Health

Depending on the synthesis process, nanomaterials could not be entirely safe. Nanomaterials that have been created by chemical, physical, and biological processes have been found to be toxic. Due to the gradual release of the chemicals used in their synthesis, nanoparticles made chemically are more hazardous. Nanomaterials formed biologically are more environmentally friendly (Elemike et al., 2019). Since different techniques and reactions are used during the synthesis of nanomaterials, the resulting materials are formed in different sizes and morphologies, giving them unique features. The physicochemical characteristics of nanoparticles (NPs) must be taken into consideration before using them in any field, since this could directly impact their potential health and environmental risks, restricting their actual potential as a beneficial entity.

Nanomaterials provide a variety of novel opportunities for sustainable agriculture. However, in some cases, harmful environmental effects of nanomaterials have been reported. It has been reported that fullerene (C60) nanoparticles are nontoxic to soil microorganisms but toxic in aquatic environments, indicating that the environment influences nanomaterials' reaction and behavior (Masrahi et al., 2014). According to a study, zinc oxide nanoparticles had more lethal effects in acidic soils compared to calcareous soils (García-Gómez et al., 2020). When ZnO nanoparticles (800 mg kg^{-1}) were applied to cucumber plants, they showed toxic effects as reported by Zhao et al. (2013). It has also been reported that CuO NPs inhibit wheat plant roots from growing (Dimkpa et al., 2020). These reports suggest that nanoparticles alter the nutrient quality of crops in several different ways.

The primary cause of NPs' toxic effects is metal NPs as they rely on the production of excessive ROS, especially by splitting ions in the biological environment (Yan & Chen, 2019). Furthermore, it also disrupts the ETC (electron transport chain) in the chloroplast and mitochondria. Carbon fixation is also affected by stress factors, which leads to photoinhibition and an increase in superoxide anion radical

O(2)(−) and H_2O molecule generation (Foyer & Noctor, 2005). Further interactions of ROS with biological components result in changes in protein compositions, lipid peroxidation, and DNA damage.

A comprehensive and holistic strategy like life cycle analysis (LCA) is necessary for deeper understanding, assessment, analysis, and regulation of nanoparticles (Salieri et al., 2018). The life cycle evaluation is a comprehensive method for determining whether the manufactured nanomaterial is environmentally safe. By quantifying their effects on an organism's environment and life cycle, LCA enables the evaluation of nanomaterials for their environmental sustainability. As a result, LCA is unquestionably advantageous for the full analysis of the nanomaterial in order to improve its enforcement and efficacy for the environmental system. Furthermore, LCA also recognizes the effectiveness of nanomaterials for the ecosystem by estimating the energy conversions and emissions created in a system. The application of nanotechnology to enhance plant nutrition and agricultural production is getting popular. However, more emphasis should be placed on the safety of these materials, as there is a fine line between toxicity and deficiency. Although there have been beneficial breakthroughs in the application of nanotechnologies in agriculture, we cannot completely rule out the possible risks related to their use. Numerous elements need to be taken into account before realizing the effective and positive utilization of NPs in the agricultural sector.

2.8 Nanobiofortification for Human Health

Selenium Selenium (Se) is necessary for the human, animal, numerous prokaryotic, and certain algae metabolism (Pilon-Smits, 2019). Se is the sole metalloid which integrated into particular proteins known as selenoproteins, some of which play critical enzymatic roles, such as selenocysteine, the 21st amino acid (Roman et al., 2014). 25 selenoproteins carry out selenium's nutritional activities in humans with selenocysteine at their active center. Selenium is required for appropriate immune system function (Avery & Hoffmann, 2018), limiting HIV progression to AIDS (Muzembo et al., 2019), and vital for effective male and female reproduction (Qazi et al., 2018, 2019) and lowers the incidence of autoimmune thyroid disease and cancers (Radomska et al., 2021; Santos et al., 2018).

The tight gap in Se deficit (40 g per day) and toxicity (>400 g per day) is a fundamental distinguishing characteristic of Se. Adults should take 55 g per day in the United States, 55 g per day in India, and 55–70 g per day in Europe.

Iodine Iodine (I) is required by the human body in order to produce thyroid hormones (thyroxine (T4) and tri-iodothyronine (T3) (Sorrenti et al., 2021). Inadequate iodine is among the most prominent micronutrient deficits, causing various clinical and social problems known as IDD. An enlarged thyroid, known as goiter, is the most common symptom of I deficiency (Zimmermann et al., 2008). As a result, chronic iodine deficiency decreases the synthesis and activity of thyroid hormone, resulting in major negative consequences on health, particularly in expectant women and their children. Furthermore, I has already been mentioned as having a significant function in postnatal improvement and brain tissue adaptability (Toloza et al., 2020). Thus, iodine deficit can thereby impact embryonic programming through the imprinting of cerebrospinal axis and then have postnatal consequences (Velasco et al., 2018). In contrast to Se, iodine in plants is mostly transported in the xylem tissue, making it simple process to biofortify plant leaves (Mackowiak & Grossl, 1999). Iodine biofortification in grain is more difficult; hence, leafy greens like lettuce, cabbage, fenugreek, and spinach can be biofortify with iodine (Weng et al., 2013). The suggested daily consumption of iodine are as follows: 90 g for 0–5-year-old children, 120 g for 6–12-year-old children, 150 g for adults, and 250 g for pregnancy and lactating (WHO, 2007).

Zinc Zinc (Zn) deficiency is a major factor for malnutrition worldwide. Zn is a crucial element to maintain good health of individuals (Calayugan et al., 2021). The importance of Zn for humans was established in 1961 (Roohani et al., 2013). Zn prosthetic groups are found in approximately 2800–3000 proteins in the human mass. Zinc is also needed for the proper functioning over 300 enzymes (Praharaj et al., 2021; Tapiero & Tew, 2003). Zn is essential for cardiovascular health, skin health, functioning of the reproductive system, nervous system, respiratory system, and endocrine system. Zn deficiency could affect almost 2 billion individuals. The effect of Zn deficiency is severe in children and pregnant women. Zn shortage is among the major causes of death in children globally, affecting more than 178 nations (Nishida et al., 2004). Zinc-deficient juveniles are susceptible to pulmonary issues, diarrhea, retardation, and low brain development (Young et al., 2014). Skin issues and dwarfism recurring infections are common in toddlers and school-aged children (Hambidge & Krebs, 2007). Adult male require 11 mg of Zn daily, while women require 9 mg. Female should take 1315 mg of Zn daily during pregnancy. Babies aged 7 months to 3 years, 4–8 years, 9–13 years, and 14–18 years should consume 3 mg, 5 mg, 8 mg, and 11 mg of Zn per day, respectively. Given Zn's importance in individual's well-being, a rising concern is enhancing Zn dietary intake via biofortification through nanoparticle-based zinc nutrients (Kapoor et al., 2022).

Copper Copper is the third most prevalent trace transition element in the humans, following Fe and Zn. It is used in human and animal metabolic processes (Bhattacharya et al., 2016). It is required as a regulator in several enzymatic processes, involving respiratory oxidation and the metabolism of Fe. The role of Cu in oxidation and reduction explains its vital role in production of neurotransmitters and pigments. Recent research has revealed that copper is an important element for optimum neuronal functioning. Copper occurs in two redox states, Cu^{+1} (cuprous) and Cu^{+2} (cupric) that are important in natural systems. Cu(I) is converted from an insoluble form to a highly solvable and bioavailable form Cu(II). However, at high exposure levels, it causes toxicity in biological organisms (Siddiqui et al., 2013). Cu ion homeostasis must be carefully regulated in order to maintain good health. A healthy adult's daily diet is estimated to contain 0.6–1.6 mg of Cu (Gutfilen et al., 2018). The lack of Cu nutrient is thought to contribute to cardiovascular and osteoporosis as a chronic dietary conditions, and its imbalances have been found in carcinoma patients. The toxicity and deficiency of copper can be inherited or acquired.

Iron Iron (Fe) is a trace element required for cell division, oxygen and electron transport, differentiation, and gene expression regulation (Piskin et al., 2022). In the human body, 70% of the iron binds to predominant protein (haemoglobin) in the red blood cells (RBCs), myoglobin, which stores oxygen in muscle cells and cytochrome system, essential in the energy generating process (cellular respiration). Anemia is the main cause of Fe deficiency and affects approximately 2 billion persons globally (Frater, 2021). The suggested daily Fe intake for men of all ages and postmenopausal women is 8 mg per day, and for pre-menopausal, it is 18 mg per day. The median dietary intake of Fe is nearly 16–18 mg per day for male and 12 mg per day for female (Institute of Medicine (US) Panel on Micronutrients, 2001).

2.9 Conclusion

Micronutrient deficiencies have serious consequences for both plant and human health. By minimizing losses from soil leaching and volatilization, nanotechnology-based approaches may aid in producing nutrient-rich foods or by assisting in the process of genetic transformation and modification of genes that are involved in the micronutrient absorption, translocation, and accumulation. An overview of the most common nanomaterial-based biofortification techniques is provided here. These methods can biofortify food crops effectively to address micronutrient deficiency in humans in a sustainable manner. In addition to these benefits, using NFs in agriculture aids in reducing pollution through controlled nutrient delivery at the appropriate time. Future research should concentrate on nanotechnology based biofortification of food crops under complete life cycle growth conditions, as well as effects of nanomaterials on crop yield and nutritional quality.

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Chapter 3
Nanobiofortification of Vegetables for Nutritive Values and Qualitative Traits

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Contents

3.1 Introduction

Agriculture acts as the primary pillar of the developing economy and provides food for a better life. The field of agriculture is currently confronting several difficulties, including unexpected climate change, soil pollution by dangerous environmental pollutants like fertilizers and pesticides, and significantly increased food demand due to a growing worldwide population (Pouratashi & Iravani, 2012). Human health

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is a significant worldwide concern, and improving it has been and continues to be the main goal of almost everyone around the globe. All environmental components, such as soil, edible plants, drinking water and air, as well as the complete sharing of microbes in the agroecosystem, have a direct or indirect impact on human health (van Bruggen et al., 2019). The focus of numerous research projects in the fields of medicine, agriculture, and industry is on how to enhance human health. Improving human health may entail providing people with sufficient and safe foods to address malnutrition and a variety of ailments. The provision of enough and safe nutrition to prevent malnutrition and fight against numerous diseases like COVID-19 may be part of improving human health.

Biofortification is a process by which edible plants can be enriched with essential nutrients for human health against malnutrition. The most important biofortified food crops include rice (de Lima Lessa et al., 2020), wheat (Shi et al., 2020), maize (Cheah et al., 2020), cassava (Okwuonu et al., 2021) and sweet potato (Siwela et al., 2020) or horticultural crops like pear (Pessoa et al., 2021) and strawberry (Budke et al., 2020) or pulse crops (Jha & Warkentin, 2020).

The major nutrients, which could be used in biofortification, may include boron (Hussain et al., 2020), copper(Grujcic et al., 2021), iron (Okwuonu et al., 2021), iodine (Dobosy et al., 2020), calcium (Pessoa et al., 2021), selenium (González-García et al., 2021) and zinc (Pal et al., 2021). Aside from minerals, edible crops can also be biofortified with certain vitamins, such as vitamin B1 (thiamine), B2 (riboflavin), B3 (e.g., niacin), B5 (pantothenate), B6 (e.g., pyridoxine), B7 (biotin), B9 (e.g., folates and their derivatives) and B12 (cobalamin), as well as vitamin C (Tiozon Jr. et al., 2021).

After the great success of the biofortification approach in the human struggle against malnutrition, a new biotechnological tool in enriching the crops with essential nutrients in the form of nanoparticles to supplement human diet with a balanced diet is called nanobiofortification. The application of foliar nanoparticles of vital nutrients (such as Cu, Fe, Se and Zn) or their nano-fertilizers in soils or waterways can result in nanobiofortification. Many barriers prohibit the biofortification of all necessary minerals for human nutrition in the nano-form utilising just edible plants.

Nanobiofortification is a new approach which helps to enrich the crops with essential nutrients to supplement human diet with a balanced diet using nutrients against malnutrition. This novel strategy has a number of benefits and drawbacks, similar to those of nanoparticle-based nutrients or nano-fertilizers (El-Ramady et al., 2020a, b, c). These nano-nutrients, like other nanomaterials, have mixed effects on the environment, the human body, and health (Malakar et al., 2021; Silva et al., 2021). Promoting crop productivity and nano-remediation of soils and water may be good aspects, but toxicity and nano-pollution may be the primary negative effects (Rizwan et al., 2021). The green synthesis of nanoparticles (NPs) could be achieved using plant extracts (i.e., leaves, roots, flowers and seeds), microbes (e.g., bacteria, yeast, fungi and algae) and biomolecules (enzymes, proteins and carbohydrates), which represent biological substrates instead of chemical as solvents and stabilizing agents to reduce the harmful nature of the product (Abinaya et al., 2021). In comparison to physical and chemical approaches, the biogenic creation of nanoparticles is "a boon" to human health and is also more practical, affordable and environmentally benign (Stephen et al., 2021). Many studies reported on the green synthesis of nanoparticles using plant extracts such as the production of S-NPs using leaves of *Ocimum basilicum* (Ragab & Saad-Allah, 2020), iron-NPs by green tea and black tea leaves (Mareedu et al., 2021), copper-NPs from *Eucalyptus globulus* and mint leaves (Iliger et al., 2021), zinc oxide-NPs from *Nilgiriantusciliantus* leaf (Resmi et al., 2021), nickel oxide-NPs from fennel (*Nigella sativa*) seeds (Boudiaf et al., 2021) and magnesium oxide-NPs from different plant extracts (Abinaya et al., 2021). These nano-nutrients, such as nano-selenium (He et al., 2021) and ZnO-NPs, can also aid humanity's fight against various diseases, notably COVID-19 (Gatadi et al., 2021).

Biofortification enhances micronutrient content in staple food crops and can be accomplished through a variety of methods including agronomic biofortification, selective breeding and genetic manipulation (Khush et al., 2012). Biofortified crop production is a cost-effective, one-time investment that provides farmers with long-term benefits. Agronomic biofortification is the physical administration of micronutrients to food crops, either directly to the soil, as a foliar spray, through seed priming or by immersing seedlings in fertiliser solutions (Dimkpa et al., 2020). The nutritional status of rice, wheat, maize, barley and sorghum has been improved by enhancing the content of Zn, Fe and selenium (Se) in edible tissues via agronomic biofortification (Ram et al., 2016). The main drawback of mineral fertiliser application is the need for repeated amendment, which is more labour- and resource-intensive, potentially causing secondary environmental damage (Bilski et al., 2012).

Plant breeding techniques and biotechnological technologies such as marker-assisted selection (MAS) can also be utilised to create desirable micronutrient-enriched plants (Mayer et al., 2008). Backcross breeding programmes resulted in the development of Zn-biofortified wheat cultivars such as "Zinc Shakti," "Zincol-2016," WB-02 and HPBW-01 (Singh & Velu, 2017). Plant-growth-promoting (PGP) microorganisms (Khan et al., 2019; Singh et al., 2018a, b, c) may also boost soil micronutrient availability and food crop bioavailability by producing chelating compounds such as mugineic acid and siderophores. Inoculation of arbuscular mycorrhizae, for example, increased micronutrient (mostly Zn) availability in soil (Madhavan, et al., 2012). Biofortification can also be accomplished using advanced methods such as genetic engineering to produce transgenic crops via direct gene transfer and genome editing, which precisely alters the target genes to produce desired genotypes. This technique maximises nutrient accumulation in edible tissues while minimising the impact on other developmental and physiological properties of economically significant crops (Vanderschuren et al., 2013). Transgenic rice (*Oryza sativa* L.) produced with high Fe and Zn can provide 30% of the estimated average requirement (EAR) for both nutrients (Trijatmiko et al., 2016). Recently, CRISPR-Cas9 has attention and reported by several workers (Chauhan et al., 2022), and CRISPR-Cas9 was used for the biofortification of β-carotene in rice endosperm. The altered rice line's endosperm was found to contain 7.9 g/g of β-carotene, identical to Golden Rice 2, and is capable of giving more than 50% of EAR for vitamin A (Dong et al., 2020). However, the expensive nature of this approach and the widespread unease toward genetically modified crops significantly limit its practical use. Importantly, nanotechnology has the potential to revolutionize agricultural systems by providing safe, easy and effective delivery of agrochemicals and may be used for the biofortification of food crops (Dimkpa et al., 2020). This article critically reviews recent progress in nanotechnology-based biofortification of vegetable crops. Further, the application of nanomaterials in vegetables, their fate and their impacts on nutritive value and qualitative traits is discussed.

3.2 The Role of Vegetables in Human Health

Plant foods constitute a significant portion of the human diet, providing the majority of the calories, minerals and bioactive chemicals required to maintain health and prevent disease. Vegetables are an important aspect of a plant-based diet, supplying dietary fibre, phytochemicals (such as vitamins and antioxidants) and minerals (Wang et al., 2017). Minerals are considered important nutrients since people cannot synthesise them and must get them through diet. Humankind evolved as a result of the dietary assumption of a considerable number of vegetables and their inadequate consumption is one of the causes of many noncommunicable diseases that are prevalent in Western civilizations. Potassium, calcium, selenium and iodine, for example, can help to maintain healthy blood pressure, bone strength, hormonal production, heart health and mental health (Schreinemachers et al., 2018). In a recent study carried out in the UK, data analysis from more than 40,000 people showed that changes in fruit and vegetable consumption may not only benefit physical health in the long-run but also mental well-being in short term (Ocean et al., 2019); besides the general population, these benefits were also observed in cancer survivors (Zhang et al., 2021). Vegetables, on the other hand, serve a crucial part in the economy, combating poverty, hunger and malnutrition, because they may be grown locally and consumed in a wide range of shapes, sizes, colours and flavours (Ogutu et al., 2020).

Nonoptimal micronutrient intake and undernutrition, also known as “hidden hunger,” can be more acute in those who maintain a restricted diet for religious, ethical or medical reasons (Sharma & Verma, 2019). Dietary reference intakes (DRI) have been established by health authorities based on recommended daily allowances (RDA) and acceptable upper levels (UL). In general, methods to address vitamin or mineral deficiencies must strive to meet the DRI for each component while not exceeding the UL (Sanahuja et al., 2013). However, the real contribution of phytochemicals and minerals to human diet is not solely determined by their concentration in specific plant tissue. Micronutrients must be liberated from the food matrix during the gastrointestinal tract’s passage, absorbed into the blood and delivered to their target tissues (Boland et al., 2014). In reality, only the percentage released from plant tissue eventually becomes available for absorption. This percentage is bioaccessible, and increasing the bioaccessibility of plant phytochemicals and minerals is a prospective target of nanobiofortification techniques to improve vegetable nutritional quality (D’Imperio et al., 2016).

3.3 Basics of Nanobiofortification

Nanotechnology makes use of a material's nanoscale (<100 nm) properties. Siddique and colleagues (2015) investigated the role of nanoparticles in plants. Because nanoparticles (NPs) have unique physicochemical qualities, such as high surface area, high reactivity, variable pore size and particle morphology, nanotechnology opens up a wide range of novel applications in the biotechnology and agriculture industries (Khan et al., 2019). Precision agriculture can benefit greatly from nanotechnology by increasing the number and quality of staple crops through biofortification (Sharma et al., 2017; Xiong et al., 2017). Nanoparticles applied as a foliar spray or in the soil can boost growth, crop output (shoot, root and yield) and in-plant micronutrient levels (Deepa & Ganesan, 2015). With the nanoparticle-assisted controlled release, nutrient availability can be maintained in a responsive manner while also minimising leaching and promoting effective nutrient accumulation in edible tissues. The proper elucidation of nanoparticles' physiological, biochemical and molecular mechanisms in plants leads to improved plant growth and development.

Humanity is confronted with several health-related issues, namely, malnutrition and hidden hunger. These issues manifest as a lack of minerals and vitamins even in those who consume a healthy amount of calories (Tiozon Jr. et al., 2021). This mineral and vitamin shortage could be remedied through biofortification. It could be defined as "biofortification is a process that enhances the bioavailable concentrations of enriched vitamins or minerals in staple diet like rice achieved through three different approaches, namely, (a) agronomic biofortification, (b) conventional breeding or (c) transgenic and gene editing approaches." Nanobiofortification refers to the use of nutrients in the form of nanoparticles to enrich edible plants for human health, as described in numerous studies on Cu, Fe, Mn and Zn oxide-NPs (Czarnek et al., 2023); ZnO-NPs (Thunugunta et al., 2018) and nano-selenium (e.g., El-Ramady et al., 2020a, b, c; Seleiman et al., 2021). Nanobiofortification is a new approach which helps to enrich the crops with essential nutrients to supplement the human diet with a balanced diet using nutrients against malnutrition. To provide grown plants with the necessary and appropriate nutrients for plant nutrition, which serves as the primary source for human health, nutrient-based nanoparticles or nano-nutrients are an important source. Engineered NPs could be used as food additives or in the food industry, such as colourants, emulsifiers, taste enhancers, artificial sweeteners, foaming and anti-foaming agents, directly for human consumption (Medina-Reyes et al., 2020). The antibacterial properties of the nanoparticles, which also come in the forms of silver (Ag), titanium oxide (TiO_2) and zinc oxide (e.g., Ag-NPs, TiO_2-NPs and ZnO-NPs), could be used in food packaging (J. Deng et al., 2020). Although many nanoparticles have been applied as nano-fertilizers or nano-pesticides, which promote crop productivity but might cause some problems in soil-plant in interfaces, particularly the over-doses (Ragab & Saad-Allah, 2020). Several studies have depicted applied engineered-NPs as nano-fertilizers (e.g., Guo et al., 2018; Farshchi et al., 2021; Madzokere et al., 2021) to improve crop productivity under many stresses (Landa, 2021) like drought (Sreelakshmi et al., 2020) salinity

(Zulfiqar & Ashraf, 2021), pollution of heavy metals (Noman et al., 2020) and biotic stress (Tauseef et al., 2021a, b). Through a variety of processes, including enhancing the antioxidant defence system, stimulating photosynthesis, and raising water, nutrients and phytohormones, these nanoparticles can benefit farmed plants under stress (Zulfiqar & Ashraf, 2021). The primary benefits of engineered nanoparticles on cultivated plants may include their potential use as agricultural agents (such as nano-fertilizers, nano-pesticides and nanogrowth enhancers), their ability to protect plants from environmental stresses (such as salinity, water shortage and drought) and their ability to reduce the accumulation and toxicity of heavy metals (Landa, 2021).

To enable the biofortification of food crops, engineered nanoparticles with desired physicochemical properties may be a safer alternative (Elemike et al., 2019). Both top-down and bottom-up methods are used to create nanoparticles. Bottom-up approaches involve chemical and biological methods, including photochemical, sono-chemical, vapour deposition, microwave, sol-gel, electrochemical deposition, spray/laser pyrolysis and atomic and molecular condensation strategies. The bottom-up approach refers to the build up of material from the bottom: atom-by-atom, molecule-by-molecule or cluster-by-cluster. Many of these techniques are still under development or are just beginning to be used for the commercial production of nanopowders. Conversely, top-down approaches involve physical methods, such as sputtering, chemical etching, mechanical/ball milling and photolithography (Arole & Munde, 2014).

Top-down approach involves the breaking down of the bulk material into nano-sized structures or particles. Top-down synthesis techniques are an extension of those that have been used for producing micron-sized particles. Top-down approaches are inherently simpler and depend either on the removal or division of bulk material or on the miniaturization of bulk fabrication processes to produce the desired nanomaterials that are biosynthesised with the assistance of microbes or plants. In this case, microbial or plant extracts convert elements, oxides or ionic/salt forms into nanoscale materials (de Brito Mateus et al., 2021). Algae, fungus, actinomycetes and bacteria are used in microorganism-mediated procedures, whereas plant-assisted methods make use of tissues like fruit, sap, stem, bark, root and leaves as well as agricultural waste (Malik et al., 2014). For the creation of nanoparticles, plant-assisted technologies also use phytochemicals (carbohydrates, proteins, terpenoids, phenolics, flavonoids, amino acids and saponins). Additionally, phytochemicals may serve as capping or stabilising agents for the resulting nanoparticles. Green methods for synthesising nanoparticles with the assistance of plants are environmentally friendly, economical, effective, quick and less harmful (Singh et al., 2018a; Baig et al., 2021).

NFs are frequently used to release nutrients into the soil in a controlled manner, which can increase the nutrients' availability to various plant organs and increase plant yield and quality (Sekhon, 2014). When compared to the same amount of traditional fertiliser, NFs are more supportive of plant development and environmental safety due to their capacity to cover a larger surface area and their effective absorption by plants. These are used in smaller amounts, which results in less leaching and fewer gas emissions into the atmosphere (Adisa et al., 2019). The effectiveness of NFs varies depending on their chemical characteristics, size and composition and mainly the crop they are applied on (Thakur et al., 2018). NFs are defined as compositions with very small sizes, usually equal to or less than a nanometer, made up of macro- and microelements like N, P, K, magnesium (Mg), calcium (Ca), sulphur (S), iron (Fe), zinc (Zn), copper (Cu), molybdenum (Mo), boron (B) and their compounds like cerium oxide (CeO2), titanium oxide (TiO_2), silver (Ag), gold and zinc oxide (Elemike et al., 2019; Dimkpa et al., 2017; Prasad et al., 2017; Mejias et al., 2021).

Three different types of NFs are being successfully used for biofortification programs:

(i) Nanoscale-coating fertilizers, where conventional fertilizers are encapsulated by nanoparticles (NPs) or intercalated in nanopores (such as zeolites and nanoclays) either to help the delivery or delay the release of a nutrient or to supplement with an additional element at a nano-level (Tarafder et al., 2020).
(ii) Nanoscale fertilisers or NFs are the NPs containing nutrients themselves that are used as fertiliser, and each particle is less than 100 nm in size.
(iii) Nanoscale additive fertilisers are traditional fertilisers enhanced with NPs of a nutrient (Mejias et al., 2021).

Nanomaterials that are utilized for vegetable biofortification can also be classified as (a) polymeric nanomaterials made up of repeated chains of molecules differing in structure and compositions (Moreno-Vega et al., 2012); (b) ceramic nanomaterials that are nonmetallic and heat-resistant nanomaterials composed of both metallic and nanometallic compounds (Fellet et al., 2021) and (c) metal nanomaterials made up of metallic compounds such as metal oxides, quantum dots, nanogold and nanosilver (Trotta & Mele, 2019). Moreover, these materials can be grouped according to their nutritional benefit to the applied plants, such as (a) micronutrient fertilizers, (b) macronutrient fertilizers, (c) plant growth-stimulating NFs and (d) nanomaterial-enhanced fertilizers (Fellet et al., 2021).

3.4 Mechanism of Nanoparticle Uptake, Translocation and Accumulation in Plants

The potency of NP uptake depends on plant species, as well as particle size, chemical nature, stability and function. It has been researched how the size of NPs affects their uptake in wheat plants; for instance, Fe_2O_3 NPs of size 8–20 nm efficiently pierced the roots and moved to the leaves (Al-Amri et al., 2020). Additionally, significant variables affecting absorption and translocation in plants include the surface area to charge the ratio and concentration of NPs. NPs with higher surface area-to-volume ratios can enter the root and leaf surface more easily (Burke et al., 2014). In soybean (*G. max*) maximum Zn (8 nm) uptake was found at 500 mgL^{-1}, whereas a reduction in Zn uptake was observed at higher ZnO-NPs concentrations, i.e.1000–4000 mgL^{-1} (López-Moreno et al., 2010). The reduction in Zn uptake might be attributed to the formation of aggregates at higher concentrations, making it difficult to pass through the cell wall pores. Other studies have revealed that nanopriming of seeds facilitates enhanced uptake of micronutrients, although the precise mechanisms of action are not known (Rizwan et al., 2019).

Foliar application of nanoparticles is perhaps the most direct way to fortify plants. Foliar uptake of NPs from the leaf surface occurs through cuticular and stomatal pathways (Lv et al., 2019). Lipophilic substances enter by diffusion in leaves via the cuticular pathway, while polar or ionic substances enter through stomatal pores (0.6−4.8 nm diameter). Therefore, NPs with size below 4.8 nm may cross through cuticular pathway. However, the uptake of NPs (with size >5 nm) likely involves other pathways of foliar uptake. In the stomatal pathway, hydrophilic substances enter through stomata pores with diameter ≥40 nm. Due to small cuticular pore size, studies generally support the stomatal pathway for NPs uptake in leaves (Wang et al., 2021). After entering into the leaf apoplast, NPs translocate to grain, fruit, stem and root via the phloem. The uptake of Fe_2O_3, magnesium oxide (MgO) and ZnO-NPs (24–47 nm size) in watermelon (*C. lanatus*) was confirmed as penetration of NPs through the stomata (Wang et al., 2013). Further, the presence of NPs in shoots and roots suggests their translocation to the roots via the phloem. Foliar uptake of ZnO-NPs in coffee plants was evaluated by X-ray micro-analysis and confirmed Zn-NPs accumulation in treated leaves (~1267 $mgkg^{-1}$ dry wt.) as compared to the control plants (~53.6 $mgkg^{-1}$ dry wt.). Importantly, both cuticular and stomatal pathways remain involved in foliar uptake of Zn-NPs; the significance of each pathway may vary (Singh et al., 2018a, b, c). Foliar uptake and translocation of Fe_3O_4 NPs from the leaf to the stem and ultimately to the root via the phloem was observed. The Fe accumulation pattern in wheat seedlings was as follows: leaves>stem>roots (Cai et al., 2020; Lu et al., 2020). Xiong et al. showed that after foliar application of CuO NPs in cabbage and $Cu(OH)_2$ NPs in lettuce, a significant amount of Cu (97–99%) was accumulated in the leaves and only a small fraction (1–3%) translocated and accumulated in roots (Xiong et al., 2017). Another study revealed that the greatest absorption of γ-Fe_2O_3 NPs occurred via foliar application as compared to soil application (Alidoust & Isoda, 2013).

Most studies on nanoparticle uptake are focused on root uptake as compared to foliar exposure, in part because nanoparticles tend to per-sist longer in soil than on leaves. The interactions of NPs in soil and subsequent uptake and translocation of NPs in roots are more complex in comparison to foliar uptake. NPs have to cross many barriers, including the root cuticle, epidermis, cortex, endodermis and Casparian strip for transport to the shoots through the xylem (Fig. 3.1). NPs applied in the soil either directly or as nano-fertilizer will initially adhere to the root surface and then penetrate into the roots via aquaporins, ion channels, endocytosis and carrier proteins. The movement of NPs across membranes via endocytosis is the most recognized route of uptake (Schwab et al., 2016). Aquaporin transporters also have a role in NPs uptake; Wang et al. demonstrated that inhibiting aquaporin activity decreased Se NPs influx by 60.4% in rice seedlings (Wang et al., 2020a, b).

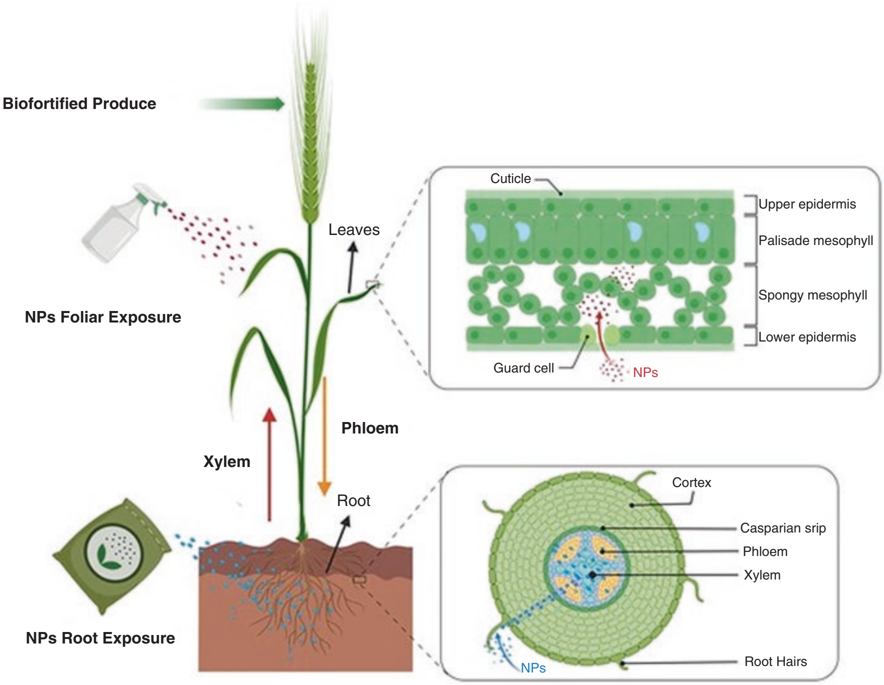

Fig. 3.1 The schematic representation of foliar and root uptake of nanoparticle-based fertilizers via the stomata and root hairs, respectively, and their translocation through the xylem and phloem. (Kapoor et al., 2022)

The surface of the root is primarily negatively charged due to the secretion of organic acids from the root hairs; this therefore promotes the adsorption and accumulation of positively charged NPs. NPs can also directly cross the root hair cuticle due to its poor development, subsequently reaching the epidermis (Schwab et al., 2016). For further translocation of NPs, both apoplastic and symplastic pathways have been demonstrated in various reports. The apoplastic pathway involves the movement of NPs through intercellular spaces; for the symplastic pathway, NPs are translocated from cell to cell via plasmodesmata (Fig. 3.1). Apoplastic movement of NPs is widely accepted due to the presence of NPs in the intercellular space of root tissues in a large number of studies (Li et al., 2016; Wang et al., 2012). Although the Casparian strip acts as a barrier to apoplastic movement, NPs may enter the xylem through the root tip (where the Casparian strips are not developed) or via junctions in the lateral root region (where the Casparian strip is detached) (Lv et al., 2015; Schymura et al., 2017). For example, the accumulation of ZnO NPs in the lateral root junction and xylem of maize has been reported. Therefore, this junction is important to the apoplastic movement of NPs into the xylem (Lv et al., 2015). The uptake and translocation of CuO NPs through the xylem and phloem in maize and rice was investigated and the authors reported the presence of endosomes having

CuO NPs, as well as particle accumulation in the intercellular spaces of root cells, xylem sap and leaves.

Hence, CuO NPs can enter the plant cells by endocytosis (followed by the apoplastic pathway) and move into the xylem with subsequent transport to different parts of the plant. Further, it was found that CuO NPs biotransform (Cu^{2+} to Cu^{+}) during translocation from roots to shoots. Cu content in treated rice plants followed the order: roots>mature leaves>stem>young leaves (Peng et al., 2015). The uptake and translocation of Fe_2O_3 NPs in maize was studied using transmission electron microscopy (TEM), and apoplastic movement of Fe_2O_3 NPs from the root epidermis to endodermis was observed, although no information about translocation to other plant tissues was studied (Li et al., 2016) (Fig. 3.2).

In higher plants metal acquisition under deficient conditions have been well categorized into two basic strategies: first strategy in nongraminaceous plants and the second strategy in graminaceous ones. Strategy I follow the metal chelates reduction at the surface of a root by metal reduction oxidase (MRO), and then the absorption of metal ions across the plasma membrane of root cells with the help of metal-regulated transporter (*MRT*) gene and later extrusion of protons and phenolic compounds in the rhizosphere which increases the solubility of metal ions on the surface of roots (Kobayashi & Nishizawa, 2012) (2A). In contrast, strategy II includes uptake of metal by plants under metal deficiency through secretion of metal-chelating phytosiderophores like mugineic acids (MA) and nicotiana amine (NA) possessing strong affinity for metal ions by forming a metal-phytosiderophore-soluble complex, which gets transported in to the root cells of the plant (Singh &

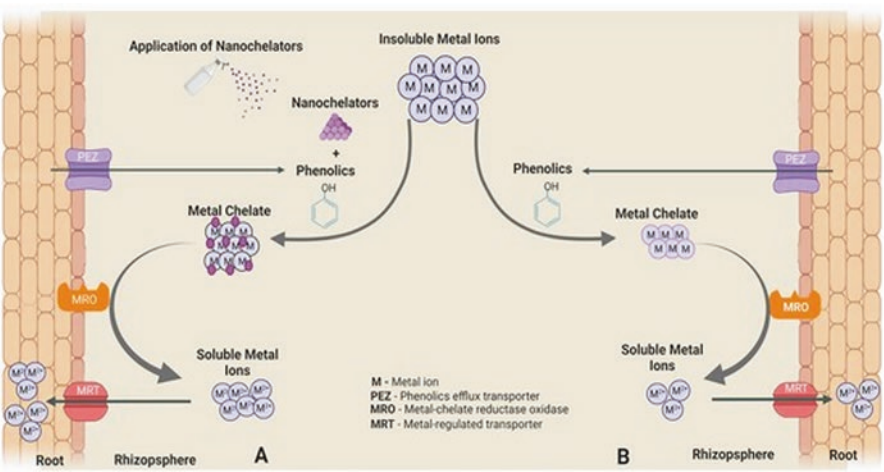

Fig. 3.2 An illustration of different strategies for metal ions transport in plants, (**a**) Nanochelator-aided strategy (strategy I) demonstrates chelating of metal ions with nanochelators for their easy uptake through metal-regulated transporters, (**b**) phytosiderophore-aided strategy (strategy II) demonstrates chelating of metal ions with phytosiderophores released by plant in deficiency conditions to facilitate easy uptake of metal ions. (Kapoor et al., 2022)

Prasanna, 2020) (2B). Sega et al. reported that the application of citrate-capped $FePO_4$ NPs significantly increases the P level to more than double in the shoot of cucumber plant as compared to bulk-treated or negative controlled plants (Sega et al., 2020). Similar treatment given in maize (*Zea mays*) plants significantly increases the P level. Fe concentration also increased subsequently in root tissues of maize and cucumber on the application of $FePO_4$ NPs, whereas lesser increase was observed for Fe concentration in the shoot of maize plant than cucumber compared to control plants. Therefore, it has been concluded that cucumber plants (strategy I species) uses $FePO_4$ NPs as a P source, whereas maize (strategy II species) uses $FePO_4$ NPs as a Fe source preferentially (Sega et al., 2020).

The mechanism of NPs uptake, translocation and accumulation in plants are still poorly understood, in part because most studies are conducted at the seedling stage (Lv et al., 2019). Therefore, additional research to understand the mechanisms of transport of NPs in plants is critical to establish their suitability in agricultural applications. For example, NPs translocated mainly through the xylem should be added to the soil, whereas NPs transported via phloem would be more amenable to foliar application (Aslani et al., 2014).

3.5 Nanobiofortification of Minerals in Vegetables

Vegetables are produced under a wide range of agronomic contexts, depending on the crop cycle, soil type or growth environment. The use of mineral fertilisers and/or improved mineral element mobilisation and solubilization in the rhizosphere are two common agronomic strategies to raise the concentration of minerals in edible organs (White & Broadley, 2009). Vegetable crops are typically cultivated in agrosystems that have a high degree of production process intensification and where fertigation, soilless cultivation and foliar fertilisation are increasingly used to deliver nutrients. These options present various ways to carry out targeted biofortification projects. When applying minerals to soil-cultivated crops via fertigation, some interference may result from the elements' plant availability (phytoavailability); therefore, selecting mineral forms and concentrations may have relevant importance (White & Broadley, 2009; Carvalho & Vasconcelos, 2013). Hydroponic system farming is one alternate method to address the low mineral phytoavailability in the soil (soilless cultivation). Soilless agriculture techniques have become widely used due to the possibility of maximising scarce water resources and growing in the lack of suitable agricultural soils. For instance, it has been found that hydroponic cultures are sometimes one of the greatest ways to boost the nutritional content of plant tissues (Wiesner-Reinhold et al., 2017; Li et al., 2017). Another option is the use of foliar fertilisation for minerals that are not easily translocated to the edible tissues, such as for crops grown in soil or for minerals with limited mobility (Niu et al., 2020).

3.5.1 Growth and Development of Vegetables

The discovery of methods for a variety of low-cost nanotech applications for improved seed germination, plant growth, development and acclimatisation to conditions constitutes the new scientific innovation platform known as nanoscience seed germination is a delicate stage in a plant's life cycle that promotes seedling growth, survival and population dynamics. However, a variety of factors, including the environment, genetic makeup, the availability of moisture and soil fertility, have a significant impact on seed germination (Fig. 3.3).

1. *Effects of Nano-TiO_2 Photosemiconductor on the Photosynthesis of Cucumber Plants.*

   The mechanism of TiO_2 semiconductor photocatalysis had similarities with artificial photosynthesis. The effects of nano-TiO_2 photo semiconductor sol on the photosynthesis of cucumber plants had been firstly reported in this paper. Anatase TiO_2 semiconductor used in the experiment was synthesized by sol-gel methods, and its effects on photosynthesis, activities of root systems and contents of leaf cell malondialdehyde had been studied after spraying different concentrations of nano-TiO_2 sol on cucumber leaves in culturing experiments. The experimental results showed that nano-TiO_2 sol could form perfectly adhesive, transparent, continuing and stable films on the surfaces of leaves by which net photosynthetic rates and activities of the root system had been significantly promoted. These results provided some scientific and technical references for the application of nanomaterials which use TiO_2 as effective ingredients in agricultural research (Ping et al., 2008)
2. *Application Research of Nanobiotechnology to Promote Increase of Vegetable Production.*

   The experiments on fertilizer efficiency on radish, cabbage, cabbage, eggplant, peppers, tomatoes, celery and leek crops were carried out over the past 2 years. The results showed that the fertilizer promoted the growth of the crops come into the market 5–7 days ahead of time and made the yield increase 20–40%. After fertilization, the white radish grew to 83 cm in 38 days, eggplant 1.2 kg in 20 days and so on. Nano-fertilizer could improve the quality of vegetables. The content of vitamin C in chilli increased 1.5 times (Jian et al., 2009).

3.5.2 Vegetable Quality

Numerous noncommunicable diseases, including cardiovascular disease, malignancies, diabetes, obesity and metabolic syndromes, can be lowered by eating enough high-quality veggies. Vegetables are a sign of a healthy lifestyle when they are included in dietary composition. The amount of dietary fibre, vitamins, minerals and phytochemicals or antioxidants in vegetables is higher.

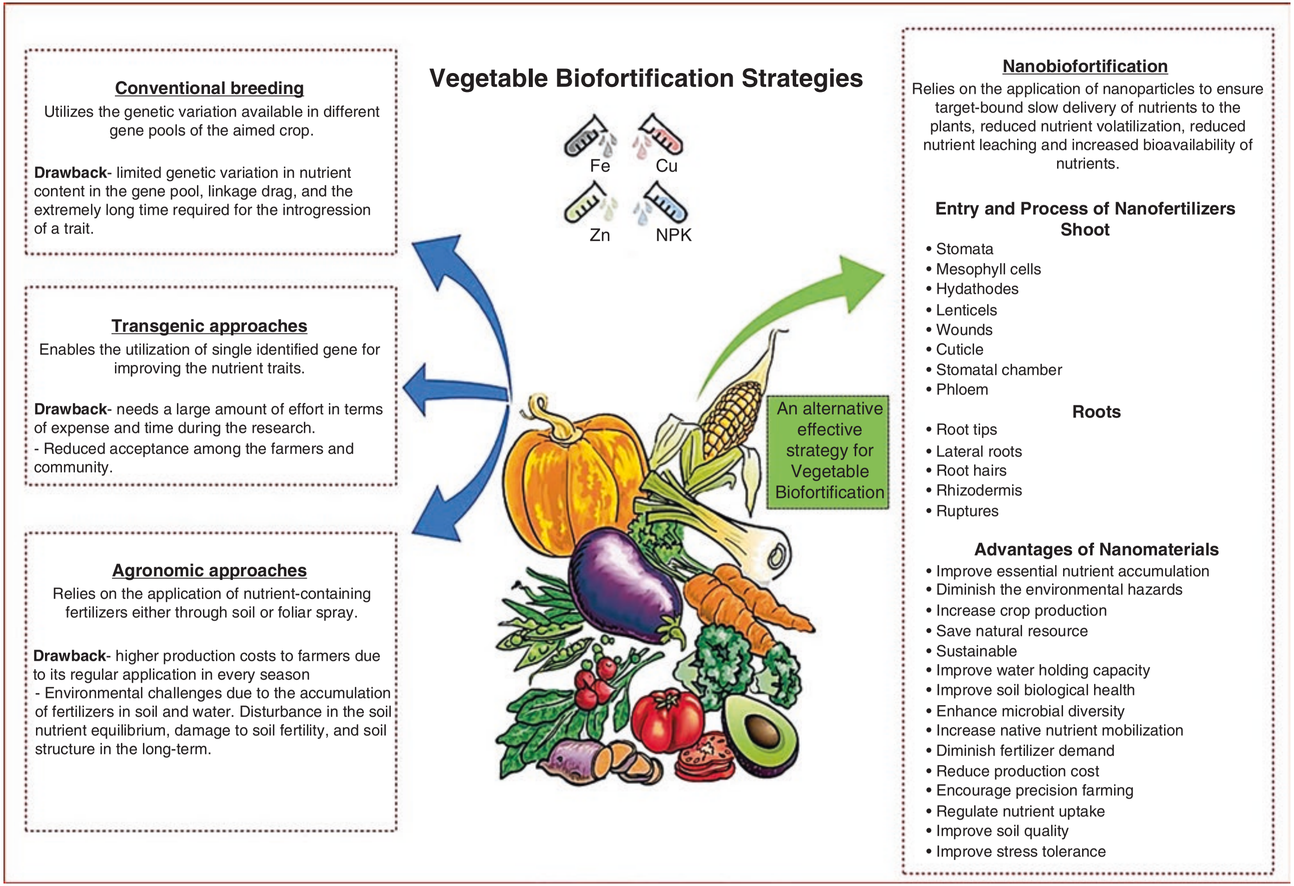

Fig. 3.3 Different strategies used for vegetable biofortification. The left side of the figure explains the vegetable biofortification strategies other than nanobiofortification and their drawbacks. The right side of the figure provides a summary of nanobiofortification including its target, entry points of nano-fertilizers in plants and the advantages of nanomaterials (Fe-iron, Zn-zinc, Cu-copper, NPK-nitrogen, phosphorus and potassium)

Cucumber's (*Cucumis sativus*) nutritional qualities are altered by CeO_2 and ZnO nanoparticles. The effects of nanoparticles (NPs) on the fruit quality of cucumbers are not well understood. This study sought to ascertain potential effects on the fruit of cucumber plants cultivated in soil treated with CeO_2 and ZnO NPs at 400 and 800 mg/kg on carbs, proteins, mineral nutrients and antioxidants. Changes in functional groups were detected using Fourier transform infrared spectroscopy (FTIR), and the distribution of nutritional elements was measured and mapped using ICP-OES and -XRF, respectively. The results demonstrated that none of the ZnO NP doses had any effect on sugars; however at 400 mg/kg, CeO_2 and ZnO NPs enhanced the quantity of starch. Conversely, CeO_2 NPs did not affect starch content but impacted nonreducing sugar content (sucrose)

Fourier transform infrared spectroscopy (FTIR) data showed changes in the finger print regions of 1106, 1083, 1153 and 1181, indicating that both NPs altered the carbohydrate pattern. ZnO NPs did not impact protein fractionation; however, CeO_2 NPs at 400 mg/kg increased globulin and decreased glutelin. Both CeO_2 and ZnO NPs had no impact on flavonoid content, although CeO_2 NPs at 800 mg/kg significantly reduced phenolic content. ICP-OES results showed that none of the treatments reduced macronutrients in fruit. In the case of micronutrients, all treatments reduced Mo concentration, and at 400 mg/kg, ZnO NPs reduced Cu accumulation. μ-XRF revealed that Cu, Mn and Zn were mainly accumulated in cucumber seeds. This is the first report on the nutritional quality of cucumber fruit attributed to the impact of CeO_2 and ZnO NPs (Zhao et al.*,* 2016).

3.5.3 Fertilizer Use Efficiency and Nutrient Uptake

Nano-Fertilisers Can Be Used to Supply Nutrients. Fertilizers that Have nanomaterials on them Are Called Nano-Fertilizers. By Holding onto Nutrients longer Due to Higher Surface Tension than Conventional Surfaces, they Increase Nutrient Availability to Plants and Increase Yield in Crops. They Increase the Efficiency of Nutrient Utilisation by Reducing Immobilisation. They Have Less of an Environmental Impact than Synthetic Fertilisers because they Produce less Agricultural Waste and Less Nutrient Runoff Due to Leaching and Volatilization. Nano-Fertilizer Improved the Plant Growth, Yield and Fruit Quality of Cucumber, and it Can Be Used as an Alternative to Mineral Fertilizers (Merghany et al., 2019). The Presence of zinc and Sulphur nanoparticles Affected the Growth of Broad Bean crop at Different Concentrations (Ghidan et al., 2020)

Micronutrient Deficits Can Be Avoided by Applying Soil or Spraying Plants with Nanoformulations of the Nutrients. The most Prevalent Type of zinc Deficiency in vegetables Is Small Leaf in Brinjal, which Results in a Variety of Physiological Diseases. Utilizing Fertilisers with zinc Oxide nanoparticles Can Help to Stimulate

Growth by Giving zinc micronutrients and Thanks to its Antibacterial Qualities. The Level of Plant Nutrients Can Be Detected with the Aid of Nanosensors. Precision Farming Could Benefit from the Efficient Use of Natural Resources like Water, Nutrients and Agrochemicals with the Aid of Nanosensor-Based Smart Delivery Systems (Rai et al., 2012). Thus, it Is Strongly Advised to Utilise Nanosensors to Increase Production by Tracking Nutrient Levels and Enhancing Plants' Usage of Nutrients and Other Resources

3.5.4 Plant Protection

Vegetable diseases and viruses can be found and diagnosed using nanosensors. Immune sensor based on gold nanoparticles was developed by Verdoodt et al. (2017) for quantitative analysis of Gram-positive bacteria. Enzymes are more likely to be activated and biomolecules necessary for plant defence are more likely to be synthesised when nanomaterials contain micronutrients like copper and zinc. A different approach to weeds, diseases and pest control is the use of nanomaterials. Silver nanoparticles are of particular interest because they have a high surface area relative to bulk silver, which results in enhanced antibacterial capabilities. Several nanomaterials act as antimicrobial agents are employed in food packaging.

Engineered nanoparticles with biocidal capabilities are the active components in nanopesticide formulations. Targeted distribution and controlled release increase the active ingredient's bioavailability to the pest. Nanopesticides come in a variety of forms, including nanocapsules, nanospheres, nanomicelles, nanogels, nanoemulsions, nanofibers, nanoliposomes and more, depending on the structure and morphology of the nanosystem (Balaure et al., 2017). The cotton leaf worm, *Spodoptera littoralis*, appears to be easier for tomato plants to control with the help of nano-silica particles, which also enhanced yield (El-bendary & El-Helaly, 2013).

Nano Pesticides Have Improved Herbicidal Efficacy and Prolonged Release, which Effectively Inhibit Weed Growth. By Reducing Leaching and Negative Impacts on Nontarget Plants, they Help Reduce Harmful Environmental Effects and Raise the Level of Safety in vegetable Production.

3.5.5 Postharvest Management

Vegetables are highly perishable hence postharvest management is important to increase shelf life and to maintain quality. Before packaging, spray Nano-Cu (0.5 ml/L) to extend shelf life. Vegetables are protected from rotting after harvest by coatings made of nanobiofilm and nanomaterials, which have antibacterial and antioxidant characteristics. Additionally, it has been shown that combining nanomaterials with conventional preservation techniques can extend the shelf life of harvested goods in a synergistic way (Xu et al., 2018). Nano chitosan-based coatings avoid decay of cucumber and other vegetables.

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Chapter 4
Nano-Biofortified Crop Plants with Zinc for Human Health

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Contents

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4.1 Introduction

Micronutrient imbalance in human dietary intake, often called “malnutrition,” is a serious global issue (Timotijevic et al., 2013), and more than half of the human populations is challenged by growing malnutrition worldwide. People may become malnourished in two ways: (i) people who have enough food resources but do not eat sufficient food and (ii) those who eat enough (overnutrition) but lack nutrient variations in their diet. In both situations, the micronutrient deficiencies affect human health, depressingly leading eventually to a rapid and/or enduring health problems, slow recovery from wounds/ailments and illness, and maximum danger of infections. These in turn cause severe negative impact on national economic development and productivity because of human health costs and human capital depletion (Ramakrishnan, 2002). According to the World Health Organization (WHO), almost 2 billion people suffer from iron deficiency, and around 750 million peoples have iodine insufficiency (Solanki & Laura, 2018). Besides this, zinc deficiency is also increasing alarmingly and has been recognized as an important dietary issue which can trigger health problem. The deficiencies of nutrients in dietary intake therefore requires urgent attention to protect deteriorating human health worldwide. In this regard, the nutrients that have attracted attention of many scientists while working on biofortification process include vitamins (Bouis & Saltzman, 2017), calcium and iron (Salgueiro & Boccio, 2013; Gillispie et al., 2019), zinc (Cakmak & Kutman, 2018; Das et al., 2020), copper (Elemike et al., 2019), selenium (Thavarajah et al., 2017), iodine (Lyons et al., 2004), dietary fiber, antioxidants (Martiniak et al., 2015), and dietary lipids (Timotijevic et al., 2013). While comparing the reports on nutrient fortification, the reports on zinc fortification is limited probably because zinc was earlier not considered as a human health threat, but now it is commonly accepted that zinc deficiency results in poor organ growth and immunological competence especially in children (Cakmak & Kutman, 2018). Sadly, the unavailability of zinc to approximately one third of the world’s population adversely affects the overall health mostly in developing countries including India, Southeast Asian countries, and equatorial African countries (Wessells & Brown, 2012). Apart from human health, Zn as micronutrient is essential in crop production systems as well. And so, the relationship between micronutrients provided to plants and crop growth has become a major focus of many research activities especially while optimizing the food crop yields (Subbaiah et al., 2016).

4.2 Importance of Zinc in Plant Nutrition and Human Health

Among different crucial plant nutrients, zinc is essentially required as micronutrients in various metabolic reactions that affect the overall growth and development of plants. For instance, zinc influences carbohydrate metabolism, gene expression, protein synthesis, phytohormone secretion, pollen formation, and the maintenance of other cellular activity. Apart from these, zinc also provides protection to plants from photooxidative damage, heat stress, and pathogen attack (Subbaiah et al., 2016; Verma et al., 2022). Additionally, zinc acts as an integral component of many enzymes, like carbonic anhydrases (CAs; EC 4.2.1.1), alcohol dehydrogenases (ADH; EC 1.1.1.1), and superoxide dismutase (SOD; EC 1.15.1.1) that influence various plant physiological activities (Prasad et al., 2012). For example, the ADH is important for crops growing under stress (Du et al., 2018) while SOD detoxify the impact of superoxide radicals and therefore, shields the membrane's lipids and proteins from oxidation (Wang et al., 2018). However, the deficiency of zinc causes leakage in membrane and hence, destroys the overall integrity of biological membranes. Also Zn deficiency impairs the photosynthetic process and significantly reduces the chlorophyll concentration and destructs chloroplast structure (Praharaj et al., 2021). Besides these, Zn also influences the water uptake process and transport of nutrients to different plant organs. Moreover, zinc has been reported to play significant role in providing a short-term resistance to heat and stress tolerance to plants (Peck & McDonald, 2010; Disante et al., 2011; Zhang et al., 2014).

In addition to their role in plant performance, Zn is considered an essential nutritional component that significantly influences human health. Daily intake of zinc needed by adult man and woman is 11 mg and 9 mg of Zn on a daily basis, respectively (Singh, 2022). Zinc acts as a cofactor or structural component of various enzymes such as carboxypeptidases (EC 3.4.16–3.4.18), carbonic anhydrase, RNA polymerase (EC 2.7.7.6), superoxide dismutase, lactate dehydrogenase (LDH; EC 1.1.1.27), phospholipases (A1 EC3.1.1.32; A2 EC 3.1.1.4; AD EC 3.1.4.4), and aldolases. Besides this, Zn is also found in all six types of enzymes, including hydrolases, lyases, ligases, isomerases, oxidoreductases, and transferases. In addition, the body cannot produce this micronutrient, but it is necessary for overall physical development, immunological function, reproductive health, and neurobehavioral activity (Krężel & Maret, 2016). Zn malnutrition is however a major threat that severely affects human health. To substantiate the ill effects of Zn further on human health, reports indicate that approximately 30% of the total human populations worldwide is facing Zn paucity due largely to their reliance on some staple foods such as cereals (bioavailability is low) especially in developing countries (Impa & Johnson-Beebout, 2012). The Zn shortage/deficiency may cause human illness such as poor physical growth, a compromised immune system, DNA damage, cancer, and increased risk of infections (Hotz & Brown, 2004; Gibson, 2012). Also, Zn deficiency impairs the development of embryos, new born, unfavorable pregnancy outcomes, delay in bone maturation, impaired appetite, hair loss, delay in cell recovery, and alteration in behavioral activity (Hotz & Brown, 2004; Solanki & Laura, 2018). Apart from these, diarrhea is the most distressing condition reported in infants, while in toddlers and school-aged children, skin issues, recurring infections, and dwarfism are common (Praharaj et al., 2021). Considering the variable roles and its deficiency in human dietary systems, it becomes obvious that human diets must contain Zn (Krebs et al., 2014; Terrin et al., 2015). Such human health problems, however, can be corrected by enhancing the intake of supplemental zinc that may effectively reduce the risk of malaria, pneumonia, and diarrhea (Praharaj et al., 2021). Acknowledging the beneficial role in human health, there is a growing interest in increasing zinc intake worldwide (Saha et al., 2017a).

Zinc biofortification in crop plants have shown great success in solving the malnutrition issues and hidden hunger due to shortage of quality foods (El-Ramady et al., 2021). Deficiency of vitamins and minerals could overcome through nano-biofortification (Koç & Karayiğit, 2021). Zinc oxide (ZnO) nanomaterials are considered multipurpose nanoplatforms for bioimaging and for drug delivery applications. The large surface area, variable surface chemistry, and phototoxic effect of nanomaterials are some of the important and useful features that enhances the success rate of nano-biofortification process. As an example, bench scale studies suggest that ZnO NPs can be highly lethal to cancer cells or pathogenic bacteria and leukemic T cells (Hanley et al., 2009; Wingett et al., 2016). Due to these properties, the ZnO NPs have been exploited as drug/gene delivery vehicles in addition to their role in cancer therapy. Studies have also shown that by augmenting the Zn and Fe level in rice (*Oryza sativa*) crops (consumed by 3.5 billion people), the micronutrient deficiencies were greatly reduced. In Bangladesh, several rice lines have been created through conventional plant breeding which reduces zinc deficiency (Van Der Straeten et al., 2020). In soil, zinc is present in five different forms: free or complexed ion, for plant uptake. Soil factors that cause zinc deficiency are high pH, sandy texture, high salt concentrations, high level of phosphorus, calcareousness, water logging, low content of organic matter, high magnesium and/or bicarbonate concentrations, and microbially inactivated zinc.

4.3 Zinc in Soil: Availability and Deficiency

In organic and mineral soils, concentration of zinc differs between 10 and 300 mg/g, while in agricultural soil, its level varies between 50 and 66 mg/g (Hafizi & Nasr, 2018). Among plant nutrients, zinc is absorbed as divalent cations (Zn^{2+}) by the root systems of plants which is available in soils in different forms that include (i) soil solution, (ii) adsorbed cation, (iii) soil carbonates and aluminum oxide, (iv) living organisms and organic residues, and (v) as matrix (lattice) of primary and secondary minerals (Solanki & Laura, 2018). The deficiency of zinc among plant micronutrients however is one of the critical soil factor that cause greater yield losses and reduces nutrient composition of many food crops (Hafeez, 2013; Rudani et al., 2018). For example, cereal cultivation in zinc-deficient soils have been found to further lower the zinc content in grain since cereal grains already have low zinc concentration (Aiqing et al., 2021). There are, however, different factors like high crop yields and intensive farming practices, minimum application of organic manures in cultivable fields, excessive use of agrochemicals such as nitrogenous (urea) and phosphatic fertilizers (DAP), and use of poor water quality for irrigation that cause a substantial rise in zinc deficiency in many cultivation systems. Such issues could be resolved by fertilizing food crops with suitable micronutrients. In this regard, ZnO and $ZnSO_4 \cdot H_2O$ or $ZnSO_4 \cdot 7H_2O$ have been found as the primary source of Zn in fertilizer fortification studies since oxide is not completely soluble in water. Different zinc forms, mode of application, efficacy of Zn fertilizers, time of application, and plant growth stage all play a note-worthy role in enriching crops with Zn through biofortification process (White & Broadley, 2011). It is also influenced by soil parameters such as pH, salinity, redox potential, organic matter concentrations, and the microbiome composition and functions (Mortvedt & Giordano, 1967; Milani et al., 2015; Barman et al., 2018). Due to the deficit of Zn in soil, there is urgent need to improve the Zn levels in soil so that the plants do not suffer from Zn insufficiency.

4.4 Importance of Zn Biofortification in Human Health

The human dietary systems around the world lack some of the vital micronutrients, for instance, Zn, Fe, Ca, Mg, Cu, Se, I, vitamins, and other important components that unpleasantly distress the human health and quality of life (Bailey et al., 2015; Kumssa et al., 2015). However, such mineral deficiency can be corrected by supplementing the animal products in human diets, through mineral supply, food fortification, and/or enhancing the bioavailability of minerals in eatable crops. The global dietary diversity, mineral supplementation, and food fortification measures have however failed to produce the desired results. Biological fortification, generally called "biofortification" among different measures, is a process that signifies nutritionally superior food crops with better bioavailability to the human populations that are cultivated employing different methods (Garg et al., 2018). Additionally, biofortification is an inexpensive, environmentally friendly and viable, and long-term option of adding enough micronutrients in staple food to overcome human malnutrition (Gomathi et al., 2017). Acknowledging the significance of biofortification process in the production of mineral-rich food crops, biofortification of crops through mineral fertilizers, and breeding crops to increase their mineral-absorbing capacity has been suggested in modern agronomic practices. While deciding the biofortification of edible crops, this is important to understand the amount of a specific minerals necessary in the human diet which determines the desired concentration for that very specific nutrient in the consumable organs of a biologically fortified crop. So the measures adopted for solving the mineral deficiency problems through biofortification and to target mineral element concentrations in edible crops require the understanding of local dietary and culinary practices. When a diet is deficient in more than one mineral element, biofortification techniques must provide all of them to the affected populations (Nestel et al., 2006). If mineral element concentrations in staple foods is improved, the delivery of minerals to the susceptible populations can be amplified proportionally in their diet, without requiring any behavioral change (Graham et al., 2007). Rice and wheat are the two important target staples which are consumed widely by more than 50% of the population worldwide. Apart from conventional biofortification method, other approaches like crop breeding, targeted genetic manipulation, and/or the use of mineral nano-fertilizers (Garg et al., 2018) offer great promises for circumventing human mineral deficiencies, thereby enhancing human health (Adhikari et al., 2016).

4.5 Methods of Zinc Biofortification

Zinc biofortification of food crops is a novel technology that can be helpful in reducing/circumventing the zinc deficiency impact on human health. The biofortification of edible crops containing Zn can be realized by adopting one or simultaneous strategies: (i) conventional plant breeding, (ii) agronomic biofortification, (iii) genetic engineering, and (iv) nano-biofortification.

(i) **Conventional Plant Breeding**

Plant breeding is a very old technique for producing the modified/new plant genotypes with maximum nutrients in food grains (Welch & Graham, 2004; Bouis et al., 2011). This is an economically and ecologically stable and safe method for growth and development of crops endowed with high nutritional food values. In traditional breeding program, seeds rich in nutrients are selected for producing crops. Here, high-yield crop varieties are bred with such seeds which results in the production of crops highly rich in nutrients and productivity. The breeding techniques are laborious, time-consuming, and expensive, yet such agronomic strategies offer a swift solution to the problems of micronutrient malnutrition.

(ii) **Agronomic Biofortification**

Agronomic biofortification, used to maximize the content of essential elements in growing plants through fertilization, improves the nutritional value of food crops (Prasad et al., 2014). Agronomic techniques, in general, involve application of fertilizers such as (i) soil application, (ii) seed priming, and (iii) foliar application. Among the agronomic management strategies, foliar spray and application of nutrients directly in soils are, however, regarded as the most common approach for improving the nutritional value of crops (Cakmak, 2008). By this method, fertilizer is used either as a soil application or can be sprayed on the leaves. For example, when iron and zinc were applied foliarly in phyto-available form, enhanced the accumulation of these micronutrients in plant tissue and edible organs that suggested the effectiveness of biofortification process (Saltzman et al., 2013). Other elements like selenium (as selenate); iodine, applied as iodide or iodate; and Zn ($ZnSO_4$) are the pivotal micronutrients which are used in agronomic biofortification practices. The application of fertilizers, often called fertilization approach, is indeed a rapid and matching strategy that helps to maintain and build a Zn pool, which later on is taken up by plants and through translocation is deposited in different plant organs (Cakmak, 2008). The agronomic biofortification strategy therefore is considered the most striking option for preventing zinc malnutrition.

Nutrient application in cereal crops has been found extremely efficacious with minerals like Zn and Se (Garg et al., 2018). For example, wheat crops which lack Zn prevent wheat products from containing adequate dietary Zn (Cakmak & Kutman, 2018). Zinc fertilizer applied to soil (soil application) in the wheat cropping system has been found to increase zinc content in grains. In contrast, foliar application of selenium enhances its concentration in grains via absorption through leaves (Garg et al., 2018; Singer et al., 2020). The successful agronomic biofortification of micronutrients depends on (i) the availability of stable and high micronutrient concentration, (ii) improvement in regular consumption of biofortified crops for the betterment of human health, and (iii) awareness among farmers about newer biofortification technology and understanding of appropriate delivery strategy.

(iii) **Genetic Engineering**

Genetic engineering (GE) is a recent and promising technique that employ pool of genes to produce new crop varieties by multiple gene transfer (high crop yield, high zinc, or other nutrient content) from one organism to another for improving its value and quality (Bouis & Saltzman, 2017). The GE strategy offers a suitable and affordable substitute to optimize the amounts and bioavailability of micronutrients in eatable crop tissues (Prasad et al., 2015). Genetically modified crops have enhanced nutritional qualities: high concentration of vitamins (provitamin A, vitamin B_6, folate, Vitamin B_9), zinc, calcium, and iron. Besides these, GM food crops are also well protected from diseases caused by bacteria, viruses, and pathogens (Kumar et al., 2020). Applying this technology, many transgenic vegetable crops have been produced which have certain desirable traits, such as better flavor, better nutrient absorbing ability, reduced bitterness, enhanced sweetness, slow ripening, high nutritional level, minimum growth time, seedless fruits, and reduced antinutritional factors (Malik & Maqbool, 2020). Additionally, redistribution of micronutrients in plant tissues, especially in the edible organ of the plants can be increased by genetic modifications. Numerous transgenic food crops, for example, tomato, brinjal, okra, wheat, rice, barley, sorghum, maize, legumes and pulses, common beans, lupines, oilseeds, and fruits, have been produced through genetic engineering (Bawa & Anilakumar, 2013; Delaney et al., 2018). Recently, the discovery of CRISPR/CAS9 (clustered regularly interspaced short palindromic repeats), ZFN (zinc finger nucleases), and TALENs (transcription activator like effector nucleases) technologies have provided solutions to various problems of conventional genomic technologies in order to increase the nutrient composition of leguminous crops (Bhowmik et al., 2021).

(iv) **Nano-Biofortification**

Zinc Nano-Biofortification of Cereal Crops

The biofortification method involves adding minerals or essential ingredients to the food crops. Biofortification of crops using nano-Zn can be inferred as Zn nano-biofortification where a nano-form of Zn is applied to enhance the overall Zn content in crops. A simple depiction of Zn nano-biofortification is presented in Fig. 4.1. Among many food crops consumed globally, cereal grains, such as wheat, maize,

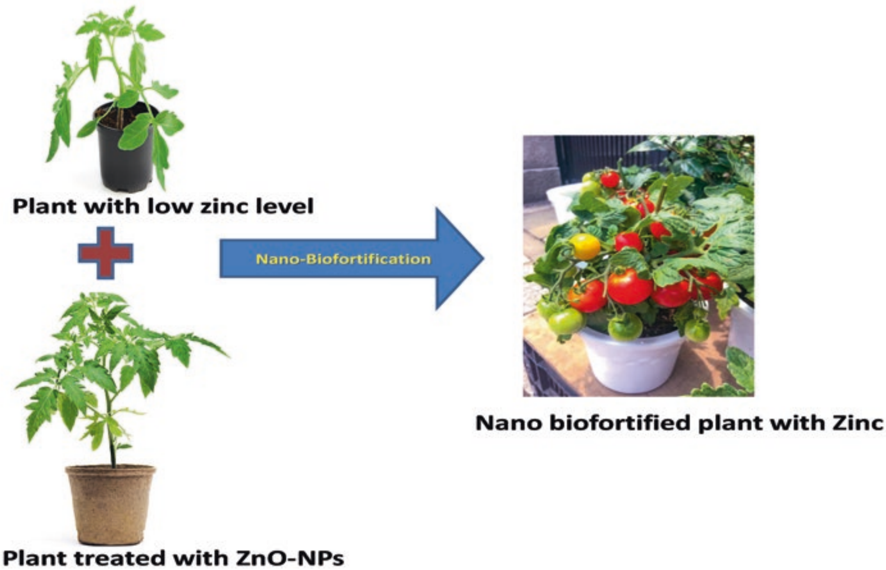

Fig. 4.1 A general illustration of nano-biofortification of Zn micronutrient to food crops (e.g., tomato)

and rice, are the most important staple food crops that provide approximately 60% of daily calories in human dietary systems (Ritchie & Roser, 2017). From nutrient point of view, the whole grain products of such crops are generally rich in carbohydrates fibers, proteins, lipids, vitamins, minerals, and phytochemicals. The traditional processing however removes majority of their nutritious components, particularly micronutrients (Laskowski et al., 2019). Due to this, the fortification of wheat, rice, and maize flour with different micronutrients especially vitamins and minerals such as iron and zinc becomes imminent (Balk et al., 2019). Some examples of the improvement of Zn content in cereal crops are presented in Table 4.1.

To address such micronutrient deficiency issues, novel ways, besides traditional fortifications approach, are urgently needed to provide stable micronutrient concentrations in grain crops. Among various approaches adopted so far, nanotechnology in recent times has been accepted as one of the most advanced and highly accurate technologies, which in agriculture can be applied to fortify crops. Nano-biofortification is a process which helps to enrich crops with vital nutrients for improving human health against malnutrition (El-Ramady et al., 2021). NPs aid in the optimization of fertilization strategies, maintaining physiological processes, for example, assimilation of micro- and macronutrients (e.g., Mg, Ca, Zn, and Na) required for biological and chemical development of plants, yield, and fruit quality (Gutiérrez-Miceli et al., 2021). Recent examples of nano-biofortification of crops are given in Table 4.2. Additionally, the nanomaterials due to many unique qualities, like high sorption capacity, ability to liberate materials gradually and in controlled manner at target sites, and a high surface-to-volume ratio (Feregrino-Perez et al., 2018), are used to manufacture nano-biofertilizers. The nano-bioformulation

Table 4.1 A few examples of improvement in Zn content of cereals

Crops	Dose	Increase (%) in zinc content	References
Triticum aestivum L.	23 kg/hac Soil application	90	Zou et al. (2012)
Triticum durum	5.35 kg/hac Foliar application	122	Dhaliwal et al. (2019)
Oryza sativa L.	0.9 kg/hac Foliar application	20	Fang et al. (2008)
Zea mays	54 kg/hac Soil application	40	Kanwal et al. (2010)

Table 4.2 Examples of some nano-biofortified crops

Nano-biofortified crops	Applied NPs	Size of NPs (nm)	Dose rate	Major experimental findings	References
Tomato	CuO NPs	42	10–250 mg/L	Improved fruit quality and antioxidant property	Hernández-Hernández et al. (2019)
Wheat	Cu NPs	17–38	25–100 mg/kg soil	Improved growth in metal polluted soils	Noman et al. (2020)
	ZnO NPs	<100	20–1000 mg/L	Enhanced high-Zn content in grains	Du et al. (2019)
Alfalfa	Cu NPs	–	80–280 mg/kg	Improved physiology	Cota-Ruiz et al. (2020)
Sunflower	FeO NPs	35–45	1–2%	Improved growth under chromium stress	Mohammadi et al. (2020)
Soybean	Fe_2ONPs	5	15–60 mg/pot	Increased crop yield	Yang et al. (2020)
Rice	Zero valent Fe NPs	20	10–80 mg/L	Improved plant growth and yield	Guha et al. (2021)
Cucumber	Nano-Se	–	25 mg/L	Improved growth under the salinity stress	Shalaby et al. (2021)
Bell pepper	Se NPs	2–20	10–50 mg/L	Increased level of bioactive compounds under salinity stress	González-García et al. (2021)
Pomegranate	Se NPs	10–45	5 L/tree at 1–2 µM	Improved fruit quality	Zahedi et al. (2020)
Pea (*Pisum sativum*)	ZnO NPs	50	100 mg/L	Efficient nutrient transfer	Skiba et al. (2020)
Eggplant	ZnO NPs		50–100 mg/L	Reduced drought resistance	Semida et al. (2021)

supplies nutrients to the plants and enhances the fertility of soil without any harmful effects on plants or soil ecosystems. For example, when seeds of peanut (*Arachis hypogaea*) were treated with variable ZnO NPs concentrations, germination of seeds, seedling vigor, and biological growth of plants increased substantially. Also, ZnO NPs improved the stem and root growth of peanut plants (Prasad et al., 2012). One of the major advantages of nano-fertilizers among many benefits is that nano-formulations can be applied in very minute amounts. Due to target-specific slow release, nano-fertilizer even at a lower rate proficiently increases the nutrient content with minimal impact onto the agro-ecosystems (Al-Mamun et al., 2021). The advantage of nano-biofortification is that it does not have problems like limitations of genetic methods and problems associated with traditional agronomic biofortification process (Thakur et al., 2018). Considering these, when applied in agronomic practices, nanomaterials, for instance, nano-fertilizers (NFs), has been found to potentially increase the (i) nitrogen metabolism, (ii) seedling vigor, (iii) formation of carbohydrate and protein, (iv) process of photosynthesis and photosynthetic pigments, and (v) movement of nutrients from underground parts (roots) to aerial region (leaves) of plants (Shang et al., 2019; Mejias et al., 2021). Also, nanomaterials have the potential to mitigate different stress conditions and to optimize crop production in derelict agro-ecosystems. Crops produced from nano-biofortification are rich in essential nutrients like vitamin A, iron, calcium, and zinc (El-Ramady et al., 2021).

4.6 Examples of some Nanoparticles Used in Crop Fortification

(i) **Iron Oxide Nanoparticles**

Among plant nutrients, iron in soil is frequently found in oxide forms such as magnetite (Fe_3O_4), maghemite (g-Fe_2O_3), and hematite (γ-Fe_2O_3). The minimal toxicity, poor availability, and superparamagnetic characteristics of iron have attracted greater attention of agronomists for its application in agronomic practices for enhancing the crop production in different agro-ecological niches. However, the iron deficiency in most food crops is due to low soil iron content. Therefore, to supply iron to growing crops, iron fertilizers/iron-chelated fertilizers are applied to solve the problem. For example, iron (γ-Fe_2O_3NP) fertilization of crops is becoming highly popular among field practitioners since it fulfils the iron demands of growing crops and therefore, enhances the iron pool of soils and crops. These nanoparticles improve many physiological and biochemical activities of plants including seed germination, root growth, chlorophyll formation, and water content (Rizwan et al., 2019; Ahmed et al., 2021a; Shah et al., 2022). Because of the dynamic property, Fe_2O_3NPs when applied to soil release iron which is absorbed by roots and then it migrates from roots to aerial tissues to improve nutritious value of crops. In this regard, the iron concentration in *Cucurbita maxima* shoots were increased significantly when plants were exposed to varying concentrations of both Fe_2O_3NPs and Fe^{3+}(Hu et al., 2017). Furthermore, in roots, workers did not find any significant difference in Fe levels between the Fe exposed and untreated control plants which suggests that the iron was also transferred and accumulated in other parts of the plants. This finding is supported by other workers who believed that nutrients (metal) can be transported to other plant organs via roots, and therefore, the root is considered as a significant route for nutrient absorption (Ahmed et al., 2021b, c). In an earlier investigation, carbon-coated iron nanoparticles have been found migrating from leaves to other parts of the plants when applied to the leaves (Corredor et al., 2009).

(ii) **Copper and Copper Oxide Nanoparticles (CuO NPs)**

Copper is an important micronutrient required by plants in a very small amounts for growth and development. Bulk form of CuO is used in agriculture, particularly as fertilizers, plant growth regulators, pesticides (fungicides, insecticides, herbicides), and soil remediation additives (Xiong et al., 2017). The excessive build-up of copper in soil, however, leads to soil pollution (Ahmed et al., 2018; Liu et al., 2018). The advent of nanotechnology offers promise to reduce environmental dangers caused by excessive deposition of copper in soil. For example, the deposition of CuO NPs in lettuce and cabbage declined the water content and overall development of crops. Because such crops are leafy vegetables, so if they are consumed, the nanoparticles deposited in their leaf tissues via food chain cause human health problems. The absorption of copper nanoparticles depends on the concentration of NPs, plant species, and environmental variables. For instance, copper nanoparticles at 1000 mg/l released 0.3 mg/l Cu^{+2}which enhanced the plant development but exhibited no toxic effect on plants. Contrary to this, some biological interactions may discharge the copper ions within tissues that could be toxic to plants (Pestovsky & Martínez-Antonio, 2017).

(iii) **Titanium Dioxide Nanoparticles (TiO_2 NPs)**

When applied as growth enhancer, TiO_2 nanoparticles at less than 4% rate enhanced the nitrogen fixation, photosynthesis, and overall growth efficiency of spinach (Zheng et al., 2005). In a similar investigation, results revealed that 30-nm-sized TiO_2 NPs when applied as nano-formulation in *Zea mays* was not translocated because the NP size was greater than the size (6.6 nm) of pore of root cells (Asli & Neumann, 2009). In another investigation, TiO_2-NP penetrated the wheat plants via root cells, while NPs did not enter wheat tissues (Du et al., 2011). Moreover, the nanoparticles were polydispersed where small-sized NPs(< 20 nm) enteredthe root cells, while the bigger-sized particles formed agglomerates in soils and hence, did not penetrate the root cells. The persistence of NPs in soil may however become dangerous because they adversely affect soil enzymes (You et al., 2018) and their associated activities leading to a considerable harmful impact on the soil-plant ecosystem.

(iv) **Cerium Oxide Nanoparticles (CeO_2 NPs)**

Cerium oxide nanoparticles (CeO_2 NPs) are a desirable nanomaterial for crop development and nutritional effects in agriculture (Cao et al., 2018). The beneficial or deleterious impact of CeO_2 NPs on crops is, however, determined by the concentrations of NPs used, the physical, biological and chemical composition of soil, and the type and species of plants under investigation. It has been observed that nanoparticles applied/sprayed in small amounts to the soil improve the development and nutritional value of growing crops. However, depending on the nature of the plants, they can have certain negative consequences at larger quantities. Still, some studies have found that greater CeO_2 NP concentrations have a beneficial effect on plants. For instance, the biological features of lettuce (*Lactuca sativa* L.) was boosted when it was grown in the presence of 100 mg/kg CeO_2 NPs, while the growth of plants was hampered at 1000 mg/kg CeO_2 NPs (Gui et al., 2015), as also observed for soybean (*Glycine max*) plants (Cao et al., 2018). During this study, Cao and co-workers found that CeO_2 NPs significantly augmented soybean photosynthetic rate at high soil moisture level. In contrast, when the soil moisture is low, the plant stomata remain closed under drought condition which in effect prevents the transpiration and CO_2 uptake by the growing plants. While comparing the effect of NPs surface charge to the absorption efficiency of plants, it has been contemplated that positively charged CeO_2 NPs are absorbed more efficiently by plant roots than the negatively charged CeO_2 NPs since positive surface charge is easily dissolved and drawn to the negative surface charge in the plant, which improves Ce transfer to the plant rhizosphere. These results are however valid only when tested at bench scale or on a small scale, but the impact of such NPs may differ when applied under real field conditions due to the variable environmental/soil conditions (Elemike et al., 2019).

(v) **Selenium Nanoparticles**

Selenium deficiency affects over 1 billion people worldwide, making its fortification or fertilization in plants essential to protect human health. Selenium can be acquired as a supplement from plant foods or meat, but the best way to fulfil human food demands of selenium is to increase its levels in agricultural food crops by spraying or adding selenite or selenates to fertilizers (Carvalho et al., 2003). Selenium is abundant in phosphate fertilizers, sewage waste, and farmyard manure (FYM), all of which can be used as a rich source of this micronutrient (Saha et al., 2017b). Selenium nanoparticles possess outstanding physical and chemical properties and are highly bioavailable. They have antibacterial, antioxidant, and other physiological activities. Due to their low toxicity, selenium has been widely used as plant and food supplements and as nanomedicines (Hosnedlova et al., 2018). Selenium enrichment can facilitate the formation of some active biomolecules like amino acids, flavonoids, glucosinolates, protein, and phenolic compounds. In selenium-biofortified tomato fruit, higher levels of flavonoids and phenolic compounds have been reported (Schiavon et al., 2013). Some examples of nano-biofortification of crops are listed in Table 4.3.

Table 4.3 Nano-biofortification of crops through different nanoparticles

Nanoparticles	Biofortified crops	Dose rate	Mode of application	Effect on plants	References
ZnO NPs	Wheat	2 g/l	Foliar	Increased Zn content up to 30%	Zhang et al. (2018)
Fe_3O_4 NPs	Wheat	20 mg/kg	Foliar	Enhanced Fe content from 40 mg/kg to 90 mg/kg	Hussain et al. (2019)
Nanochelated B, Zn, and Si	Wheat	2 g/l	Seed priming	Increased the protein content	Ahmadian et al. (2021)
Nanochelated nitrogen	Wheat	240 kg/ha	Soil	Increased grain protein by 69%	Astaneh et al. (2021)
ZnO NPs	Maize	8 kg/ha	Foliar	Enhanced grain Zn content by 82% increase	Umar et al. (2021)
Fe_2O_3	Peanut	1000 mg/kg	Soil	Fe and Zn content in roots and shoots increased	Rui et al. (2016)
$MnFe_2O_4$	Barley	125–1000 mg/kg	Hydroponic	Mn and Fe content in leaves increased	Tombuloglu et al. (2018)
Nanochelated Fe	Rice	2.5 g/l	Foliar	Increased Fe and Zn concentration by 25% and 50%, respectively	Fakharzadeh et al. (2020)

4.7 Zinc Biofortification of Vegetable Crops

(i) **Tomato**

Tomato (*Solanum lycopersicum*) belongs to the family *Solanaceae* and is generally rich in lycopene, vitamin C, lutein, phenolic compounds, and carotenoids. The quality and metabolite synthesis of tomatoes are affected by plant nutrient status of soils and growing conditions. Like other crops, zinc also influences the synthesis of enzymes and proteins involved in gene expression, biochemical reactions like, protein and carbohydrate metabolism, phytohormone regulation, defense against abiotic stress (e.g., heat stress), and photooxidative damage, and provides resistance against biotic stresses, for example, infection in tomatoes caused by pathogens (Solanki & Laura, 2018).

The impact of NPs on tomatoes has been variable. As an example, foliar spray of ZnO NPs in a study significantly enhanced the length of underground (roots) and aerial parts (shoots), dry matter accumulation (biomass), leaf area, photosynthetic pigments (chlorophyll content), and other photosynthetic features of tomato plants grown with/without salt stress (Faizan et al., 2021). In a related experiment, the engineered NPs applied as nano-fertilizers or nano-pesticides have shown significant improvement in the quality and productivity of crop under stresses like drought (Adisa et al., 2019), salinity, and biotic stress (Athar et al., 2020). Results revealed that the soil application of biosynthesized ZnO NPs (at a concentration of 25 ppm) in tomato reduced the number of flowers and fruits, but the foliar application considerably enhanced the concentrations of Mg, Ca, and Na in tomato fruits (Gutiérrez-Miceli et al., 2021). Likewise, the selenium-based biofortification on tomato plant showed enhanced antioxidant activity when plants were infected with fungal pathogen, *Alternaria solani* (Quiterio-Gutiérrez et al., 2019). Moreover, selenium and copper at concentration ranging between 10 and 20 mg/l resulted in the highest yield and improved the fruit quality of tomato plants (Hernández-Hernández et al., 2019).

(ii) **Brinjal**

Brinjal (*Solanum melongena*), also known as eggplant or aubergine, is an easily cultivated plant belonging to the family *Solanaceae*. Brinjal fruit is rich in nutrients, while other parts are used as medicine. Eggplant contains maximum amounts of vitamin C, B_6, potassium, and other minerals that helps in maintaining high blood pressure, high blood sugar level, weight loss, and cardiac problems.

During cultivation of eggplant, sweet paper, and tomato, the use of zinc-based fertilizer has been found to facilitate growth and productivity. Ordinary zinc-based fertilizer in the form of $ZnSO_4.7H_2O$ has low Zn use efficiency (1–5%). Therefore, the use of ZnO-based nanoparticles that increases crop production and nutrient use efficiency (NUE) of plants proficiently is recommended for optimizing the eggplant production. Studies have revealed that the biofortification of eggplant with fertilizers consisting of Zn, Se, and Fe mitigates the oxidative damage caused by an enhancement in the level of reactive oxygen species (ROS). In order to protect plants from oxidative stress, the ROS processing enzymes also called as antioxidants like superoxide dismutase (SOD: EC1.15.1.1), glutathione reductase (GR: EC 1.6.4.2), catalase (CAT: EC 1.11.1.6), glutathione peroxidase (GPX: EC 1.11.1.9), dehydroascorbate reductase (DHAR:EC 1.8.5.1), monodehydroascorbate reductase (MDHAR:EC 1.6.5.4), ascorbate peroxidase (APX: EC 1.11.1.11), glutathione S-transferase (GST: EC 2.5.1.18), and peroxiredoxin (PRX: EC 1.11.1.15) are secreted in distinct sites of plant cells (Amira et al., 2015; El-Saadony et al., 2021). In other studies, the ZnO NPs applied foliarly provided the protection to field grown brinjals against drought stress. Foliar spraying of ZnO NPs was found suitable for plant response under real field conditions due to its environmental friendliness as compared to soil application which may show toxicity, as reported by Semida et al. (2021). Researchers have also recommended the application of mycosynthesized ZnO NPs as nano-bioformulation to alleviate *Fusarium* wilt disease of eggplant as an alternative to fungicides vis-à-vis to promote the growth parameters and metabolic activities (Abdelaziz et al., 2022).

(iii) **Okra**

Okra (*Abelmoschus esculentus*), commonly called as ladies' fingers, is a flowering plant whose green seed pods are used as a food. The flowers, fruits, and seeds of okra contain catechins, polyphenolic compounds, flavanols, and many minerals (Elkhalifa et al., 2021). The phytochemicals of okra has shown therapeutic and

Table 4.4 Nutrients and health benefits of selected vegetables

Vegetable crops	Nutrients	Health benefits	References
Tomato	Vitamin B_1	Reduces cholesterol and blood pressure	Bhowmik et al. (2012)
Tomato	Lycopene	Antioxidant activity	Bhowmik et al. (2012)
Okra	Polysaccharide	Prevents from gastric ulcers	Messing et al. (2014)
Okra	Riboflavin	Overall good health	Petropoulos et al. (2018)
Okra	Flavanoids	Anticancer	Elkhalifa et al. (2021)
Okra	Vitamin A	Improves eye sight	Gemede et al. (2015)
Okra	Glutathione	Detoxify liver	Das et al. (2019)
Brinjal	Iron	Antenatal anemia	Fraikue (2016)
Brinjal	Glycoalkaloids	Anticancer	Gürbüz et al. (2018)
Brinjal	Chlorogenic acid	Anti-inflammatory	Plazas et al. (2013)

nutraceutical activity, and therefore they are used as medicines and as food crop to enhance human health (Elkhalifa et al., 2021).

Studies have shown that okra plants treated with phytosynthesized ZnO nanoparticles promotes shoot and root length as compared to plants treated with bulk ZnO under salt stress conditions (Alabdallah & Alzahrani, 2020). The impact of various levels of nano-iron and zinc-chelated fertilizer has been investigated using okra plants infected with *Meloidogyne javanica* (root-knot nematode). The results demonstrated a significant reduction in the number of eggs, galls, and egg masses/root system and the reproductive factor of *M. javanica* on okra plants, relative to uninoculated test plants (Rostami et al., 2021). Furthermore, zinc oxide nanoparticle has been reported to reduce the harmful effects of chemotherapeutic drugs. Some of the important nutrients found in tomato, okra, and brinjal and their health benefits are listed in Table 4.4.

4.8 Factors Affecting the Success of Zn Biofortification

The effectiveness of zinc biofortification of edible crops depends on many factors: mode of biofortification; physical, chemical and biological properties of soil; plant genotypes; concentration of applied nutrients; and culture practices. These factors are briefly explained in the following sections.

(i) **Mode of Nutrient Biofortification**

Among different modes of nutrient application, foliar application of nano-nutrients to crops is considered a better option than soil application because soil has variable physicochemical properties, such as (i) high or low pH, (ii) salinity/

alkalinity, and (iii) nutrient build up in soil. Also other problems which may influence the overall growth and yield of biofortified crops determine the success or failure of Zn biofortification of food crops. The method for preparing nutrients especially the biological synthesis for biofortification is most preferable due to its eco-friendliness and low toxicity.

(ii) **Soil Properties and Plant Genotypes**

Soil has different physical and chemical properties that may reduce or enhance the nutrient solubility, nutrient absorption by roots, and bioavailability of Zn in soil. Soil texture, pH, moisture, and organic matter regulate the solubility of Zn in soil and its transport toward plant roots (Cakmak, 2008). Of these factors, soil pH plays a vital function in biofortification of crop plants by influencing the solubility, diffusion, and bioavailability rate of Zn movement toward growing crops. However, at low pH, the bioavailability of Zn is very low (Solanki & Laura, 2018). Apart from physicochemical features of soil, the plant genotypes and their photosynthates greatly affect the biofortification process. Among plant organs, the root is the first organ that comes in direct contact with soil constituents and absorbs nutrients from the soil, and then such nutrients are transported to different aerial organs. Prior to entry inside plants, these nutrients cross the concentric layers of epidermis, root cortex, and endodermis. Differentiation of endodermis and its developmental plasticity prevents nutrient translocation across plants. Additionally, the absorption mechanisms involving ion channels and transporters of the root plasma membrane also preclude the transport of nutrients across plants.

(iii) **Concentration and Release of Applied Nutrient**

Appropriate doses of nutrients or nanoparticles should be applied in test crops for nano-biofortification. Higher concentration of applied nutrient sometimes may cause toxicity to plant health, natural ecosystem, and to humans when consumed via food chain (Jaishankar et al., 2014; Sedlacek et al., 2020). Nano-biofortification offers many advantages, but this also has limitations like nanoparticle-based nutrients or nano-fertilizers (El-Ramady et al., 2021). However, when applied at recommended dose rates, nano-nutrients optimize crop production (Shang et al., 2019). The gradual or sustained discharge of nano-fertilizers in soil is a promising approach for efficient nano-biofortification (Tarafder et al., 2020), while nano-encapsulated fertilizer helps in controlled and steady release of nutrients for longer duration (Madzokere et al., 2021). Coated fertilizers promote agricultural sustainability by enhancing the nutrient utilization efficacy and alleviating ecological problems (Zhang et al., 2021). Several chemicals, for example, chitin, cellulose, keratin, polyamino acid, and starch, are used as green bio-based coating materials. Such materials are reasonably cheap, renewable, and are capable of releasing nutrients in fertilizers in a controlled manner. Nano-silica and organosilicon are yet other super hydrophobic bio-based polymer, which improves the release properties of biomaterials having poor discharging ability (Zhang et al., 2021).

(iv) **Culture Practices**

Some of the agronomic practices including multiple cropping practice adopted by plant growers (farmers) are simple and easy that help to alleviate the nutrient deficiency of edible plants. Strategies like growing two or more crops in proximity often termed "intercropping," for example, growing dicots and gramineous species simultaneously, has also been used to upsurge the nutrient content (nutritive value) in crops.

4.9 Conclusion and Future Perspectives

Recent advances in nano-biofortification of crop plants have provided solutions to the problems of zinc deficiency to combat malnutrition and to protect peoples facing acute food hunger. Development in agricultural research focuses on production of biofortified crops for the betterment of human diet. Plant-based food items not only provides essential nutrients and energy but also some micronutrients. With the help of different methods of biofortification, malnutrition and deficiencies of nutrients have been reduced in many countries, particularly in developing and underdeveloped countries especially due to programs that focus on food crops. The use of nano-enabled technologies or nanomaterials for crop improvement is still at infant stages. Much research is directed toward achieving the sustainability goals by developed nations which also involve the use of nano-enabled fertilizers under field conditions. As it is widely known that Zn is an essential micronutrient for healthy growth of plants, the Zn nano-biofortification of crops has proven to be efficient over other conventional and non-nano-strategies applied till date. Though it is widely evident that appropriate doses of nano-Zn or other nanoparticles that also directly or indirectly promote Zn accumulation in plants, reduces the Zn deficiency in crops. However, the impact of environmental factors and/or other variables including plant species and type of nanoparticle on the absorption and consequent internalization of Zn in plant tissues cannot be denied. Therefore, to maintain adequate amounts of Zn in plants using nano-biofortification method, all biotic and abiotic parameters should be concerned, and strategies should be specific to a particular crop since growth conditions and nutrient requirements of crops are variable. Another challenge is to make zinc nano-biofortification of crops common for populations which are at risk. Farmers, stakeholders, institutions, and policy makers should make efforts to commercialize nano-biofortified crops so that this nutrient-rich food product becomes available to millions of people worldwide.

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Chapter 5
Nano-Biofortification: An Environmental Health Overview

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Contents

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5.1 Introduction

Food insecurity and malnutrition are prominent issues in this century, as per the population survey the world's population continues to increase and would be about 9.6 billion people by the end of 2050 (UNDESA, 2015). Ensuring that the Earth has enough food that is nutritious too will be a difficult task because the soil is losing its fertility due to human's overexploitation activities for the greed of higher production. This perpetually disturbs the production of crops and could lead to hunger, malnutrition, and also affect environmental health. Environmental health is a great worldwide issue, and it is directly and indirectly linked to all environmental elements (e.g., soil, edible plants, drinking water, and air) with absolute sharing of microbes in the agroecosystem (Van Bruggen et al., 2019). In the present situation, agriculture has been facing an extensive range of challenges, including unpredictable climate change and contamination of soil with various harmful environmental pollutants, such as fertilizers and pesticides (Pouratashi & Iravani, 2012). Thus, there is a need of developing alternative traditional ways of crop production technologies that could increase agricultural productivity with the required nutrients in a sustainable manner without affecting environmental health and support a pollution-free environment (Subramanian & Tarafdar, 2011). New high-impact technologies such as nanotechnology and biofortification now offer new prospects for boosting agricultural productivity, enhancing food quality and nutritional value. Along with the increased production, maintenance of ecological biodiversity plays an essential role in maintaining the delicate balance of the environment and food production as it strengthens agricultural resilience toward environmental stress. More specifically, the biodiversity for food and agriculture comprises livestock, crops, forestry, and aquaculture systems by which human beings are sustained. For numerous animals and plants, oceans and forests are home, and the major world population withstands on them for food, fodder, fuel, and fiber. Thus, any matters that could cause ecological disequilibrium significantly affect food security (Rice & Garcia, 2011).

The introduction of nanotechnology-based nanomaterials revolutionizes modern agriculture practices. Nanomaterials have high reactivity due to their large surface area-to-volume ratio, long-lasting effect, and are required in very small amounts to reduce the cost of production and eliminate the high risk of environmental health issues. Recent agriculture makes use of recent nanotechnologies to contribute enormously to food security and healthy nutrition by linking with biofortification. Nanotechnology, as applied to agriculture, is bridging the gap in nutrient loss and fortification of crops. Biofortification is one such practice that allows the development of crops which are rich in nutrients at comparatively lower cost with increased availability to the human population. It also increases variability in the present nutrient content in the crops. Basically, this can be achieved through agronomic practices, conventional breeding, or biotechnology-based approaches to increase the nutritional quality of crop products (Stein et al., 2007; Athar et al., 2020). Biofortification-based nutrition enhancement techniques provide nutritious food to poor or undernourished people (White & Broadley, 2009) in their daily routine. Farmers are using this science in the nano-regime to boost the quality and quantity of agricultural production. Nanoparticle (NP)-based biofortification is an important novice strategy of nutrient fortification of crops in tackling malnutrition with environment benign method.

5.2 Nanotechnology

"Nano" comes from Greek word "nanos," which means dwarf. In 1959, R. Feynman's concept of nanotechnology was first discussed in his talk "There is plenty of room at the bottom," and Norio Taniguchi uses the term nanotechnology for the first time in 1974. According to the National Nanotechnology Initiative (NNI), nanotechnology is the manipulation of matter with at least one dimension sized from 1 to 100 nm (1 nm = one billionth of meter = 10^{-9}). Unusual various physical, chemical, and biological properties can emerge in materials at the nanoscale, and the properties of these materials may differ in important ways from the properties of bulk materials and single atoms or molecules. Nanotechnology involves imaging, measuring, modeling, and manipulating matter at this small length scale. Through NPs as a seed treatment, soil application and foliar application can improve the nutrient status of plants.

5.3 Why Look at Nanotechnology for Agricultural Inputs and Biofortification?

Nanotechnology has revolutionized agriculture systems (Dimkpa & Bindraban, 2016; Manjunatha et al., 2016; Rai et al., 2013) due to the elegant delivery structure for agrochemicals which is safe, target bound, and has an easy mode of delivery. Due to their high surface area-to-volume ratio, nanomaterials are more effective than most conventional fertilizers and other agrochemicals and also allow slow release which promotes efficient nutrient uptake by the crops. Nanomaterials exploit the nano-porous surfaces of the plant parts on plant surfaces. With encapsulated NPs, nano-micronutrients increase the efficiency of applied materials, restoration of soil fertility and plant health, and reduction of environmental pollution and agro-ecology degradation (Manjunatha et al., 2016).

There are deficiencies associated with the conventional fertilizers which are used for biofortification, as most of the nutrients are lost out through leaching losses and they go further to pollute the underground water aquifer. So, chemical fertilizers lead to environmental pollution such as greenhouse gas emissions and hypoxia and these problems. Hence, the exploration for substitutes for the problems use of nano-fertilizers (NFs), for biofortification (Suppan, 2013) has provided the slow release properties of the nutrients, which minimizes leaching of the nutrients among other interesting properties. NFs are more beneficial to conventional fertilizers as they can triple the efficiency of the nutrients, reduce the requirement of chemical fertilizers, grow drought- and disease-resistant and nutrient crops, and are less hazardous to the environment. NPs may not be initiated activity instantly to be taken up by plants; rather a series of reactions ranging from oxidation and recombination may take place to provide the plants with the right micronutrients. So, for biofortification nutrients in the nanoscale appear to be an interesting option to accumulate nutrients in the biomass and which help to bridge the gap of nutrient deficiency. Furthermore, NFs could be designed in such a way as to address particular deficient nutrients in plants.

5.4 Synthesis of Nanomaterials

There are two approaches used for synthesizing nanomaterials, the top-down and bottom-up approaches (Fig. 5.1). Top-down methods are physical means, which are expensive and consume a lot of energy and time. This approach involves the breaking down of the bulk material into nanosized structures or particles and is an extension of those that have been used for producing micron-sized particles. Top-down approaches are inherently simpler and depend either on the removal or division of

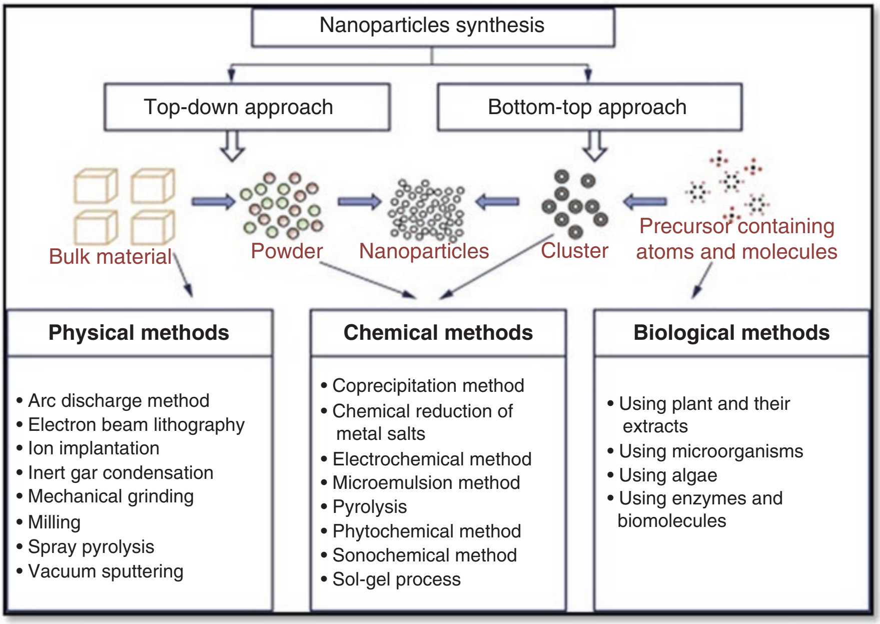

Fig. 5.1 Methods for the synthesis of nanoparticles

bulk material or on the miniaturization of bulk fabrication processes to produce the desired structure with appropriate properties. The biggest problem with the top-down approach is the imperfection of surface structure.

While bottom-up approaches have been providing the potential of creating less waste with even surface materials production and hence this approach is the more economical. The bottom-up approach refers to the build-up of material from the bottom: atom-by-atom, molecule-by-molecule, or cluster-by-cluster. Many of these techniques are still under development or are just beginning to be used for the commercial production of nanopowders. In this approach, the production of wet chemical or biological means is preferred by most researchers. Top-down methods include mechanical/ball milling, photolithography, sputtering, chemical etching, etc., while bottom-up approaches involve physical, chemical, and biological means and include sonochemical, microwave, photochemical, vapor deposition, sol-gel, chemical and electrochemical deposition, atomic and molecular condensation, spray, laser pyrolysis, etc. (Murty et al., 2013).

For green nanotechnology, the biological synthesis method served to emerge as an eco-friendly method for the production of materials and aqueous solvents, saves time and energy, and is cost-effective, sustainable, and nontoxic. The ultimate objectives of green nanotechnology are to reduce the environmental and human risks associated with the production of other methods. Biological means of synthesis consist of the microorganism-mediated method and plant-assisted green nanotechnology. More focus and interest have been given to the use of the plant-based method for the synthesis of nanomaterials. Plants are considered natural reservoirs for various components, such as flavonoids, phenolics, terpenoids, carbohydrates, proteins, saponins, and acids, that have the potential for reducing, stabilizing, and capping metal or metal oxide NPs as well as functionalizing carbon-based nanomaterials. This method provides NPs that have interesting characteristics including small sizes, eco-friendly nature, biocompatibility, low toxicity, simple reaction procedures, and enhanced surface morphologies with unlimited applications.

5.5 Biofortification

For humans, the primary source of nourishment is plants, and furthermore, the quality of food determines the health of the majority of the people. Most of the people consumed regularly high-quantity staple foods, which become the foremost part of a normal diet in a community. So, among the poor and rural communities, there is high correlation between staple foods and nutrition of their consumers, and these people hardly ever have other sources of nutrient supplementation that cause deficiencies of micronutrients, vitamins, and minerals. These have become a global issue with serious adverse effects (Hossain & Mohiuddin, 2012; Wimalawansa, 2013; Das et al., 2013; Hwalla et al., 2017; Burchi et al., 2011). To combat nutrient deficiencies, several methods have been postulated like diversification of diet and the use of industrial fortified products and drugs (Datta & Vitolins, 2016). Sharma et al. noted that these strategies have not been fully positive effects on the economic level of the people and social content. Another approach is the consumption of diverse food sources, which is suggested as a sustainable solution, but it is unaffordable to the poor community. Fortification of food nutrients by industries for nutrients has not been very successful, except for iodized salt. Biofortification is a concept of increasing the nutrient content of food crops during their cultivation in various ways (Bouis et al., 2011). The uniqueness of this method is helpful for combating micronutrient deficiencies, and it is inexpensive and available for everyone in daily routine food material because biofortified crops are staple foods widely consumed by many people and do not incur extra cost. Otherwise, foods such as animal products and vegetables are high in vitamins and minerals but are very expensive and not affordable to poor communities. Mostly the poor people spend their income on staple foods as a source of energy (Sharma et al., 2017).

Biofortification is the development of food crops that fortify themselves during cultivation which is the first and foremost agricultural tool being employed to address micronutrient malnutrition worldwide with the concept of enhancing the nutritional content of crops. For the development of biofortified crops, various strategies include agronomic, breeding, and biotechnology methods (Stein et al., 2007).

5.6 Biofortification of Crops

Biofortification means growing varieties of crops that are rich in minerals, vitamins, and micronutrients. A typical example is the development of a new variety of rice varieties rich in zinc. With biofortification, mass availability of better nutrient-rich food is definite. The target of the biofortification technique is to produce staple crops at low cost that are sustainable and have high nutritional value, able to reduce the side effects of micronutrient deficiencies. They offer the rural consumers the ability to obtain nutrient-rich foods within the community with daily routine food. There are different biofortification techniques: agronomic biofortification, conventional breeding, and nutritional genetic modification (Fig. 5.3) (Stein et al., 2007).

5.7 "Nano" Forms Used in Biofortification Programs and Their Types

Nano forms are extensively used for the precise release of nutrients into the soil that can ultimately elevate the availability of nutrients to different plant organs and also lead the improvement in its yield and quality (Sekhon, 2014). Due to their capability to cover an extensive surface area and their effective absorption by plants, nanomaterials are more supportive of plant development and ecological safety as compared to the equivalent amount of conventional fertilizer. These are applied in smaller quantities, causing reduced leaching and gas emissions to the atmosphere (Naderi & Danesh-Shahraki, 2013; Manjunatha et al., 2016; Adisa et al., 2019). The efficiency of nano form materials differs according to their composition, size, chemical features, and especially the crop for which it is used (Thakur et al., 2018). Nanoparticles are explicated as structures of very small size usually equal to or less than a nanometer in size, comprising of macro- and microelements, including N, P, K, magnesium (Mg), calcium (Ca), sulfur (S), Fe, Zn, Cu, Mn, boron (B), nickel (Ni), molybdenum (Mo), and their compounds, such as cerium oxide (CeO_2), titanium oxide (TiO_2), silver (Ag), gold, zinc oxide (ZnO), iron oxide (FeO), carbon nanotubes, aluminum oxide Al_2O_3, etc. (Elemike et al., 2019; Dimkpa et al., 2017; Prasad et al., 2017; Mejias et al., 2021).

Three diverse types of nanomaterials are being successfully used for biofortification: (i) nanoscale-coating fertilizers where conventional fertilizers are encapsulated by NPs or intercalated in nanopores (such as zeolites and nanoclays) either to help the delivery or delay the release of a nutrient or to supplement with an additional element at the nano-level (Golbashy et al., 2017; Kottegoda et al., 2017; Borges et al., 2019; Tarafder et al., 2020); (ii) nanoscale additive fertilizers where conventional fertilizers are supplemented with NPs of a nutrient; and (iii) nanoscale fertilizers or NFs are the NP-containing nutrients themselves that are directly used as fertilizer, and each particle is less than 100 nm in size (Mejias et al., 2021).

5.8 Agronomic Biofortification

Biofortification of crops by agronomic method with micronutrient fertilizer is envisioned as a fast and easy way out of the insufficiencies of these essential minerals in soils and plants. These methods use the strategy of fertilization to improve the status of micronutrients in cultivated food crops. In agronomic biofortification, to improve the micronutrient content of crops, White and Broadley (2009) recommended the use of phytoavailable micronutrient fertilizers, routine correction of the soil alkalinity, crop rotational methods of planting, and strategic introduction of symbiotic soil microorganisms. In recent, micronutrient content of crop diminutions occurs even when the yield is high, because of continuous mining of these nutrients without replenishment by especially high-yielding varieties (Zulfiqar et al., 2019; Moreno-Vega et al., 2012; Trotta & Mele, 2019). Therefore, Dimkpa and Bindraban (2016) guided that the success of any biofortification program will depend on satisfactory available micronutrients in the soil for plant absorption or supplied externally through micronutrient fertilizer because of the complex interaction required in transporting nutrients from the soil to edible portion of the crop. The improvement of agronomic fortification depends on the type of fertilizer, methods of application, packaging, and the crop developmental stage during application (Kottegoda et al., 2017; Trotta & Mele, 2019).

Recently available, conventional fertilizers are easily lost through leaching and pollute the environment, which is a major challenge. So various fertilizers and agrochemicals have low use efficiency by plants because of leaching loss, fixation, microbial degradation, photolysis, and volatilization (Chattha et al., 2017; Basavegowda & Baek, 2021). As such, amounts of these inputs are generally lower than the minimum effective doses that reach the crops. So, frequent applications are required to attain maximum yield. This pollutes the environment, including underground water sources. However, they overcome the problems and sustainably produce biofortified crops with high nutritional values requiring nanomaterials that have a high surface area, slow-releasing nature, long-lasting effect with a small amount that reduces the cost of production, and are healthy for the environment. Nowadays various organic, inorganic, and biofertilizers are availed. The components of NFs may include zinc oxide NPs (ZnONPs), silica, iron, titanium dioxide, ZnS/ZnCdSe core-shell quantum dots (QDs), InP/ZnS core-shell QDs, Mn/ZnSe QDs, gold nanorods, Al_2O_3, TiO_2, CeO_2, and FeO (Wojtyla et al., 2016). The success of using nanomaterials as fertilizers depends on the size, concentration, composition, chemical properties of nanomaterials, and species of plants (Khan et al., 2020). The linking up of biofortification with nanotechnology is needed in the vast field of knowledge of field on nano-agriculture for efficient crop production. There are three methods for agronomic biofortifications: (i) nano-biofortification via seed priming, (ii) nano-biofortification via soil fertilization, and (iii) nano-biofortification via foliar fertilization (Fig. 5.3).

5.9 Nano-Biofortification via Seed Priming

Seed priming is a pre-germinative improvement method that promotes the early emergence of seedlings by controlling metabolic activity during the first few days of germination when the plant is under drought stress. By accelerating pre-germinative enzyme activation, increasing metabolite production, repairing damaged DNA, and controlling osmosis, seed priming ensures more rapid germination (Jisha et al., 2013). This method improves the plant's potential for nutrient uptake and translocation and also enable the plant to deal with any abiotic harsh condition (Sundaria et al., 2018). The increase in nutrient uptake and accumulation also stimulates uniform germination with an increased germination rate (Ibrahim, 2016; Hussain et al., 2019a, b; Singh et al., 2018; Yadav et al., 2018). In the case of fortification of crops through conventional methods viz seed priming, fertilizer and chemicals application cause wastage of materials but by using nanomaterials, nutrients deliver more efficiently in small quantities and reduce wastage of cost and pollution which were done by the agrochemicals. Various nanomaterials such as ZnO, dextran (DEX)- and dextran sulfate (DEX (SO_4))-coated ZnO, $ZnSO_4$, and FeO are mostly used. For biofortification, the type of nanoparticles to be used that depends on the targeted objective as the surface chemistry influences the distribution of NPs within the plant and the growth of the tissue, and the biomass and response of the plant may also vary according to both the concentration and the type of treatment (Munir et al., 2018). The increase in the Zn and Fe concentration of shoots, roots, and grains of plants has been reported in wheat (Munir et al., 2018) with reducing accumulation effects of the toxicity of heavy metals such as cadmium (Cd) by using NFs of Zn and Fe. Seed priming with NPs can be considered as a promising method of wheat Zn and Fe nano-biofortification not only in the normal growth conditions but also in the Cd-stressed growth conditions and can also solve the problem of reduced germination rate of wheat seeds up to a certain extent.

5.10 Nano-Biofortification via Soil Fertilization

Various studies reported the increase in staple food nutrient content through soil application of NFs. The soil application of Fe- and Zn-mediated NFs not only fortifies food grains but also reduces some toxic substances by reduction of that element uptake and making plants abiotic stress tolerance (drought tolerance) (Khan et al., 2019). Furthermore, due to the slow release of NPs, nutrients stay in the soil and can be used for the upcoming season's crops. Other than chemically synthesized NPs, "green NPs" which are biologically synthesized achieve great attention nowadays due to their less toxicant effect and environmentally friendly nature along with their cost-effectiveness. Green NPs such as FeO, developed from bacterial strain *Pantoea ananatis* RNT4, when applied to wheat plants via soil application co-ameliorates the effect of salinity and cadmium stress and also biofortifies the wheat plants with macronutrients N, P, and K. Similarly, bacteria *Shigella flexneri* SNT22 is used to synthesize biogenic copper NPs and that regulates the Cd stress by reducing the movement of Cd from soil to plants and simultaneously increase the concentration of macronutrients like N, P, K, and Ca (Noman et al., 2020). At higher doses of 200 ppm, ZnO NPs have been found to be less or nontoxic for the plants and also elevate the increased biomass and yield (Du et al., 2019). Dimkpa et al. (2020) reported the collective effect of soil application of ZnO nanoscale and bulk particles, organic fertilizer, and drought on the accumulation of minerals in wheat grains. Results revealed that the bulk ZnO and nano-ZnO increase grain Zn content by 23% and 39%, respectively, as compared to control, while the addition of organic fertilizer can increase this content up to 94% under drought conditions which suggested that organic fertilizers along with the nano-ZnO can be successfully applied in wheat biofortification programs, especially in water-deficient growth environments (Dimkpa et al., 2020), and these strategies provide new ways toward the sustainable agriculture. Soil application of nanochitin, which is retrieved from the hydrolysis of shrimp chitin, has a significant positive effect on the wheat growth and yield, and an increase of 22.1%, 10.3%, and 5% in Zn, Fe, and protein content of multi-spike wheat and an increment of 27%, 32%, and 33.4% in Zn, Fe, and protein content of large spike wheat were, respectively, obtained by treating wheat plants with 6 mg/kg of nanochitin (Xue et al., 2017).

5.11 Nano-Biofortification via Foliar Fertilization

The foliar application of various nano-fertilizer such as Fe_2O_3, iron chelate, iron sulfate, ZnO, Si-based fertilizers, and many other bio-fertilizers are use and found to be more effective for increasing nutrient content (Ghafari & Razmjoo, 2013). Foliar application of nano-fertilizer ensures the reduced passage of NPs to soil and water, thus reducing the environmental pollution. The study revealed that the effect of foliar application of nano-ZnO fertilizer on the growth of wheat increased 20% grain protein content and also alleviate photosynthetic pigments along with reducing the leaching of ZnO into the soil as compared to the conventional Zn fertilizer foliar spray (Sheoran et al., 2021). For Fe biofortification in wheat grain, the foliar application was reported to be more efficient than soil application and reduce the grain Cd concentration under Cd-contaminated soil (Hussain et al., 2019a, b). In foliar application, a lesser amount of NPs is required as compared to soil application (Adrees et al., 2021; Khan et al., 2019). Foliar application of ZnO NFs for Zn accumulation in leaves increases the metabolic process of plants (Li et al., 2018a, b). In foliar application, direct absorption of various NPs occurs through the leaf cuticles of plants and their movement across the leaf epidermis via stomata; their release in the apoplast and then adhesion to the mesophyll cells increase the accumulation of substances in plants (Zhu et al., 2020; Read et al., 2020) along with that also increase the growth of the plant, reduce toxic substances, and make plants withstand dormant condition (Read et al., 2020; Adrees et al., 2021).

Chitosan (CHT) is the deacetylated form of chitin and the second most important biopolymer after cellulose on earth. The efficiency of foliar application of zinc-complexed chitosan NPs (ZnC NPs) as a nano-micronutrient carrier for wheat biofortification has not only enhanced the durum wheat grains with 27–42% Zn in zinc-deficient growth conditions but also enhances its translocation to both leaf and seeds and inhibits the nutrient loss to the soil. Moreover, the development of Zn fortify plants are developed by using ZnC NPs, and Zn utilization efficiency is improved by providing the materials to plants at the right time, in the right doses, and at the right place (Dapkekar et al., 2018). ZnC NP increases the expression of the genes related to metal homeostasis, including the Fe- and Zn-regulated transporter-like proteins that show a significant relationship with the grain Zn content (Deshpande et al., 2018). Various applied foliar nutrients such as Zn, Fe, and Si affect the gene expression and protein synthesis in plants, and this might be a reason for the increased protein content (Cakmak, 2000) (Fig. 5.2).

5.12 Conventional Breeding

In traditional plant breeding programs, staple food crops such as cereals, legumes, and oilseeds with high micronutrient content are selected, purified, and multiplied (Manjunatha et al., 2016) or by using other methods traits transferred from wild

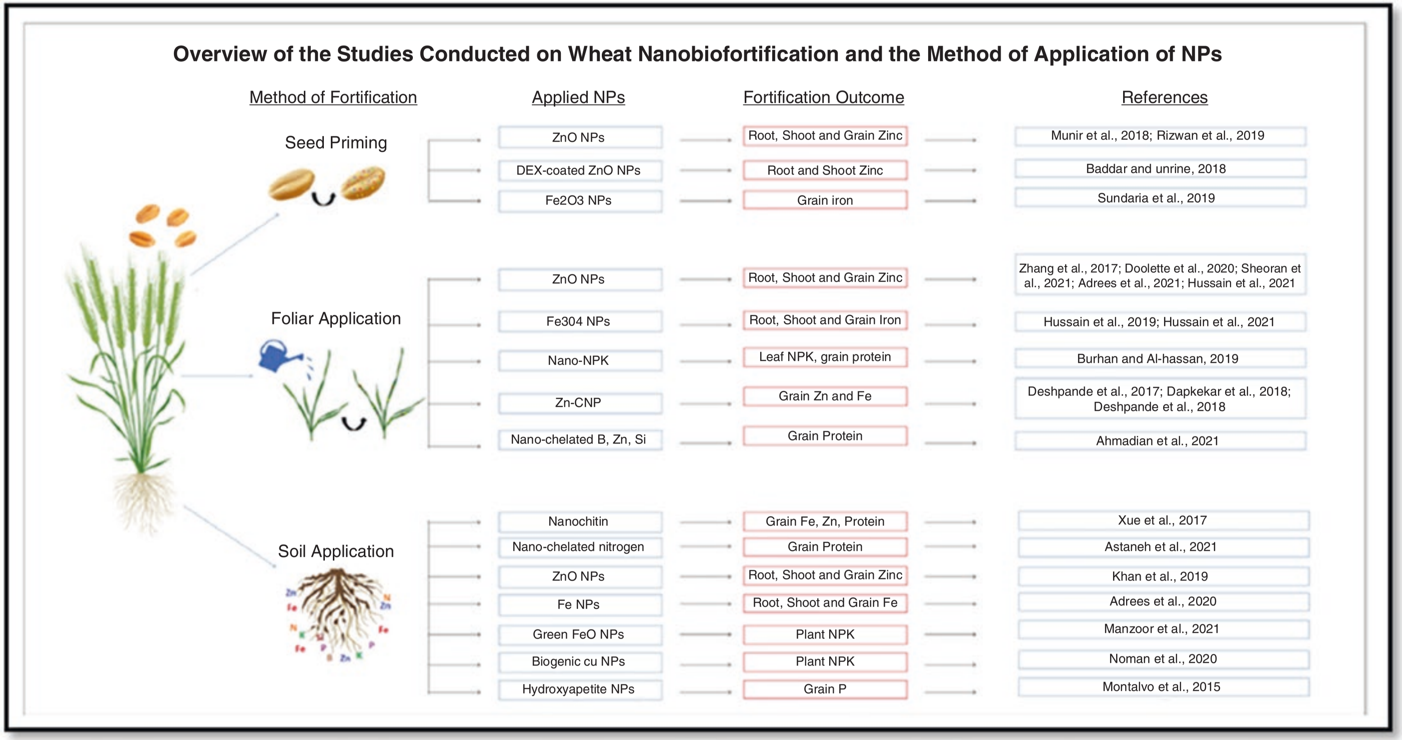

Fig. 5.2 An overview of the different methods used for wheat nano-biofortification, types of applied nanomaterials, and their outcome in the form of fortified nutrients (Abbreviations in the figure: NPs, *ZnO* zinc oxide, *dex* dextran, Fe_2O_3 ferric oxide, Fe_3O_4 ferrosoferric oxide, *NPK* nitrogen, phosphorus, potassium, *CNP* chitosan NPs, *B* boron, *Fe* iron, *Zn* zinc, *Si* silicon, *P* phosphorus, *Cu* copper). (Khan et al., 2021)

relatives or other varieties to cultivated ones. Breeding of crops is principally committed to increasing micronutrients and vitamin content in the common food crops (Adisa et al., 2019). Conventionally produced biofortified plants have more acceptance than those from gene modification. In India, LQPM 1 and 2 varieties of quality protein maize have been well accepted and can be seen as interesting examples of conventionally bred biofortified crops. Although plant breeding is the most practiced sustainable method in fortification (Elemike et al., 2019; Prasad et al., 2017; Mejias et al., 2021), it is clear that not all micronutrients can be enhanced in crops through conventional breeding in a very short period of time.

5.13 Nutritional Genetic Modification

To address the world's agricultural challenges, genetic engineering of crops has provided unique ways of modification since the knowledge of DNA level and agricultural biotechnology level are required. In genetic engineering to develop micronutrient-rich crops, infusion of genes from the wild or another organism is pursued which is not done in the conventional plant breeding approach. A study reported that improving the Fe, Zn, and Se content of crops by utilizing the plant's genetic makeup and applying biotechnological process could solve nutritional insufficiencies in human foods, but it is an expensive approach and requires lots of time (Dimkpa et al., 2017). But once the crop is developed by using this technique there are no more resources required to be invested again in each generation because the traits are now inherited and express themselves. In agronomic biofortification, in soil low or lacking in these essential micronutrients, it becomes difficult for the crops to obtain enough micronutrients. Moreover, these genetically modified micronutrient-rich crops may not be adopted by many.

The development of new genotypes with high micronutrients is a long-time scheme, and genetically modified micronutrient-rich crops may not be adopted by many people. Resulting from these restrictions, agronomic biofortification is a substitute mechanism to increase micronutrient content with the help of NF in staple food in a sustainable manner (Fig. 5.3).

5.14 Entry of Nanoparticles and Their Effect on the Nutritional Status of Plant

The nutritional status of crop plants depends upon the method of application of NPs and has a great effect on the extent of accumulation of a particular nutrient in staple food plants. However, the reason behind this is not completely understood. Thus, various application methods have different modes of uptake, absorption, and translocation of NPs in plants. The uptake and translocation of nanomaterials pass

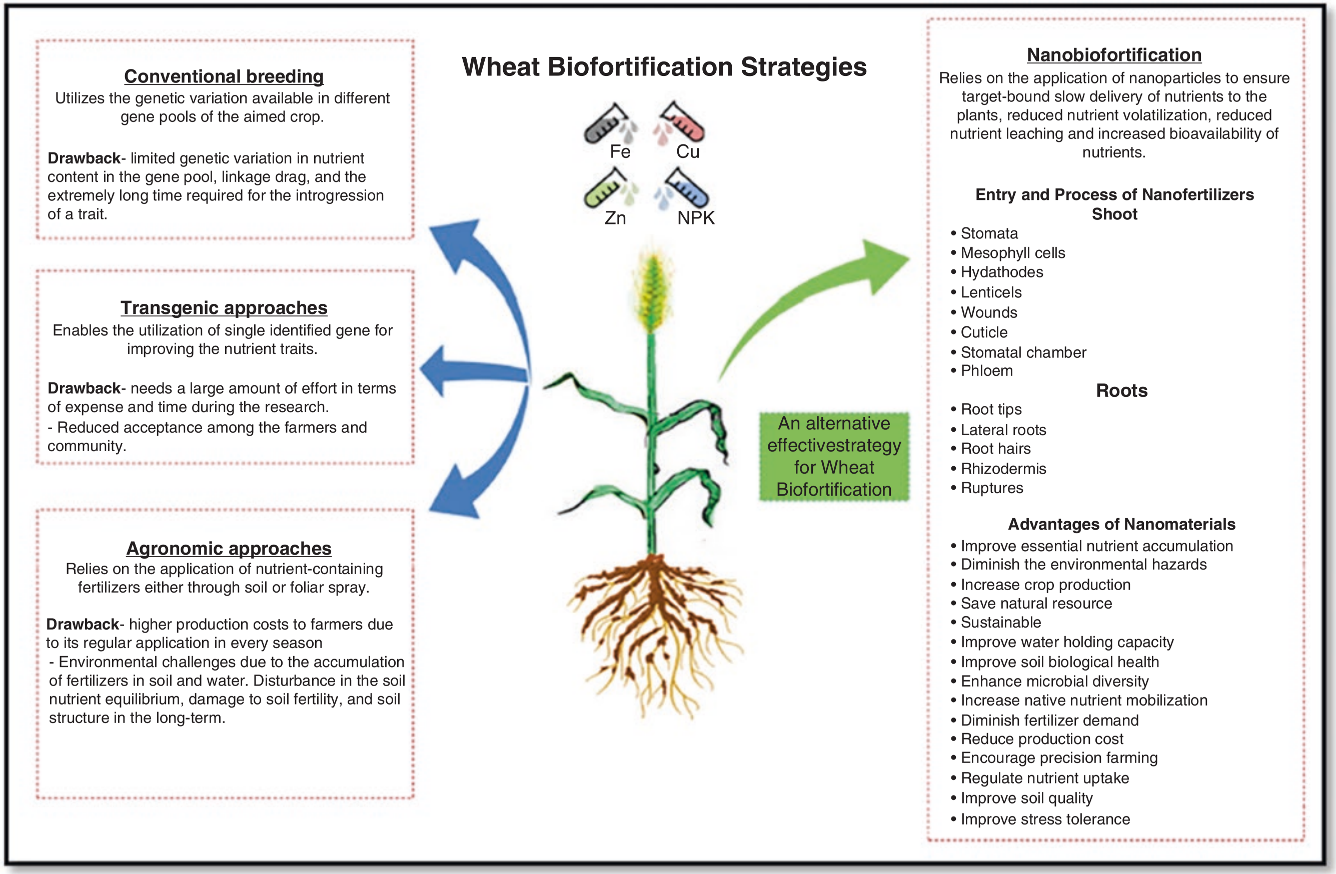

Fig. 5.3 Different strategies other than nano-biofortification and their drawbacks. The right side of the figure provides a summary of nano-biofortification including its target, entry points of nano-fertilizers (NFs) in plants, and the advantages of nanomaterials (*Fe* iron, *Zn* zinc, *Cu* copper, *NPK* nitrogen, phosphorus and potassium). (Zulfiqar et al., 2019; Elemike et al., 2019; Feregrino-Perez et al., 2018)

through different root and shoot tissues that have specific size exclusion limits and thus, act as barriers (Wang et al., 2016). But it has also been observed that some NPs can enter and translocate by making some structural changes or by forming new larger pores (Wang et al., 2016; Schwab et al., 2016). Foliar application of nutrients is considered more effective as compared to soil application because in soil application the properties of soil may hinder the plant bioavailability of nutrients.

The foliar application provides a more sustainable strategy for the accumulation of micronutrients by providing a slow release and controlled delivery of ions into leaf tissue that prevent frequent applications. Cuticle pores and stomata are considered the main pathway for entry of NPs. Zhu et al. (2020) observed stomata as the main route of entry of ZnO NPs into wheat leaves on foliar application and found increasing the zinc content in wheat. Foliar ZnO NPs are found to be increasing the zinc content in wheat and show grain Zn accumulation in the crease region, aleurone layer, and embryo (Doolette et al., 2020). The application of NPs in the soil showed higher shoot Fe concentrations. Likewise, seed priming with ZnO and Fe NPs before sowing showed results that increased the Fe-Zn concentration and also reduced harmful substance like Cd concentration in roots, shoots, and grains. As mentioned here there are three different main methods including seed priming, foliar application, and soil application that have been used for the application of nanomaterials to crop plants.

5.15 Nano-Biofortification in the Light of Cost-Effectiveness and Environmental Health

Nano-biofortification applications decrease the cost of production for farmers, and when applied in appropriate quantity at an appropriate time, these can optimize resources and minimize inputs in reference to "precision farming" and also improve environmental health. Conventionally, for agro-biofortification, fertilizers are applied several times during a growth period by farmers that increase the cost of production and that can deteriorate soil and water sources (Fellet et al., 2021) and most of the applied fertilizer is wasted due to leaching, resulting in less efficiency, less bioavailability, and repeated application in a season to plants (Achari & Kowshik, 2018). Nano-capsulated agrochemicals are designed in such a manner that they hold all essential properties such as effective concentration, time-controlled release in response to certain stimuli, enhanced targeted activity, and less eco-toxicity with a safe and easy mode of delivery, thus avoiding repeated application. So, NFs are efficient enough to overcome conventional biofortification problems when applied in large-scale field programs.

1. Apply a proper dose of nanomaterials: The higher dose of nanomaterials sometimes may cause toxicity for cultivated plants, and pollution of soil and groundwater causes consequent toxicity for humans when these plants will be consumed

by them. So the proper dose of application of nutrients must be identified before biofortification.

2. Method of nutrients application: From the various study observed, foliar application of nano-nutrients is better than soil application particularly when the used soil has some difficulties like high or how pH, salinity, and others. The biological nutrient method of application is preferable due to its low toxicity and eco-friendly.
3. There are many methods for biofortification, but more eco-friendly and less harmful methods to the ecosystem are identified as seed priming using engineered nanomaterials, which may be considered as a good pathway to improve malnutrition (Kah et al., 2019; De La Torre-Roche et al., 2020; Acharya et al., 2020) and biofortification through seed priming achieved by soil and foliar application.
4. Slow release or controlled nano-fertilizers are promising methods (Guo et al., 2018; Yu et al., 2021). For the slow and sustained release of nutrients over an extended period of time, nano-encapsulated conventional fertilizers are the best approach (Madzokere et al., 2021).
5. For sustainable agriculture, the use of coated fertilizers might alleviate nutrient utilization efficiency and reduce environmental problems like sulfur-coated urea (Zhang et al., 2021). For the green nano-biofortification approach, many bio-based coating materials such as keratin, chitin, poly-amino acid, cellulose, and starch could be used as green bio-based coating materials. These methods are considered renewable, low-cost, and have the capacity to control the release of nutrients in fertilizers. Nano-silica and organosilicon as modified superhydrophobic bio-based polymers are considered promising tools for improving the poor release properties of bio-materials (Zhang et al., 2021).

5.16 Challenges of Nano-Biofortification

Because of organoleptic problems, not all micronutrients that fall short of contents like magnesium and potassium are suitable for fortification. Furthermore, NPs are very small; problems can actually arise from the inhalation of these minute particles, much like the problems a person gets from inhaling minute asbestos particles. Sometimes NPs cause chemical hazards to edible plants after treatment with a high concentration of nano-silver, and nanomaterial-generated free radicals cause DNA damage in living tissue. Nanomaterials can disperse into soil, water, and atmosphere; NPs can also bond more strongly with pollutants and transport them through soil and water.

In the success of using nanomaterials as sources of biofortification on the plants, some other factors, such as the size, concentration, composition, and chemical properties of nanomaterials (Thakur et al., 2018), are also important, and that huge information on the fields of biology, biotechnology, material science, and engineering is crucial to the development of novel technologies needed to expand the field on nano-agriculture for efficient crop fortification. Foliar agronomic biofortification can lead to leaf tissue injury due to the release of high amounts of ions into leaf tissue that can be locally phytotoxic. For biofortification nanotechnology is very expensive and developing it can cost a lot of money. It is also pretty difficult to manufacture, which is probably why products made with nanotechnology are more expensive.

5.17 Conclusions

Hidden hunger and malnutrition are considered one of the most significant problems threatening human health worldwide (WHO, 2007). Most of the global population does not have access to fruits, vegetables, and animal products consisting of micronutrients and vitamins necessary for proper nutrition. In most developing countries, people do not have access to a proper diet consisting of fruits, vegetables, and animal products which consist of micronutrients and vitamins necessary for proper nourishment. For fulfilling the basic needs of food, the human community exploits natural resources.

Nano-biofortification has been emphasized as a practical and cost-effective strategy for delivering essential micronutrients to undernourished people in the form of a regular diet. By using various techniques of nano-biofortification, the concentration of minerals and vitamins in staple food is widely increased without using transgenic techniques, so it is more easily accepted by humans on a global scale. Nano-biofortification techniques are more sustainable and low-cost and not only improves understanding of different mechanisms in plants for increasing the yields and nutritional value but also helps in controlling diseases and regulating abiotic stresses. With these benefits, the use of NPs for biofortification also reduces environmental pollution with the precise delivery of nutrients at the appropriate time in a small amount with high efficiency.

In recent, nano-biofortification could not be thoroughly applied in field-scale programs, although applying the results derived from small experiments to the fields could largely facilitate food security, nutritional development, and a sustainable environment simultaneously. Furthermore, to apply the most environmentally friendly, biocompatible, and less toxic forms of nanoparticles for biofortification, required biological nanomaterials based on green nanotechnology can be chosen. For green nano-biofortification, various plant organs such as stems, leaves, roots, barks, and fruits can be utilized for synthesizing. Nano-biofortification mainly ensures sustainable food availability, a less polluted environment, and improved nutritional health for the world.

5.18 Future Directions

Nutrient security, food availability, and sustainable agriculture are the important sustainable improvement goals of the twenty-first century. Thus, nanotechnology is the greatest tool to harness the advantages of biofortification in a sustainable manner without compromising soil productivity. In the ecosystem, during the production of crops, many processes occur such as adding plant nutrients to the soil which may improve the production and nutrient level of food, so knowledge of new nanotechnology helps in biofortification. The uniqueness of NPs like their quantum sizes and surface area has increased application in many fields but may also have some toxic effects on the environment when used as fertilizers, pesticides, nano-delivery tools, food packaging, etc. So, further research is required to define the toxicity level of NPs which will give a better picture of potential nanotechnology.

Plants may absorb the required materials and leave the rest in the soil, and it is not clear whether the toxic materials are absorbed or become nontoxic, and, regardless, the toxic component would still be part of the ecosystem. To clear all doubts and apply the tenets of nanotechnology toward sustainable agriculture and green chemistry there is need of development of eco-friendly materials for nano-biofortification which are eco-friendly-plant origin NPs and have fewer toxic effects on plants and other organisms like green NPs. Before using NPs, it is very essential to assess their biosafety level before using them in the fields (Behboudi et al., 2018).

Due to additional intervention, nanomaterials may also lead to adverse effects and cause serious issues to health, plants, and the environment. Therefore, regulatory bodies around the world have realized the risk associated with the usage of nanomaterials (Kaphle et al., 2018). So, more research should be done to explore the mode of action of NPs, their interaction with biomolecules, their impact on the regulation of gene expression, and their effect on biofortification in plants. It is imperative to balance between the efficacy and toxicity of nanomaterials before using such materials (Thorley & Tetley, 2013). For that appropriate and strong regulatory guideline must be developed based on sound research to utilize the full potential of nanotechnology in agriculture, food, and environmental sciences.

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Chapter 6
ZnO-NP-Based Biofortification to Enhance Crop Production with Micronutrient Enrichment to Combat Malnutrition

Abhishek Singh, Vishnu D. Rajput, Neha Chakrawarti, Karen Ghazaryan, Tatiana Minkina, and Sakshi Singh

Contents

6.1 Introduction

As of now, it is clear that nearly two billion people across the globe are suffering from a zinc nutritional deficiency (Prasad, 2008). Many major disorders like dysregulation of homeostasis and immune function, oxidative stress, apoptosis, and ageing are the result of zinc deficiency. Zinc supplements in the diet have been shown to play a major role for immunity development

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against Covid-19 (Giacalone et al., 2021). The major cause of deficient dietary Zn is directly linked to low level of zinc in the soil (Tabrez et al., 2022). This is the major reason why poor and developing countries need to enhance the bioavailable Zn content in staple food grains (Zhao & McGrath, 2009).

Biofortifying crop is a long-term strategy to address human health issues and reduce hunger. In recent years (Table 6.1), there has been a lot of discussion about using nanotechnology to strengthen crops in human societies (El-Ramady et al., 2021). Nano-biofortification of crops is possible through techniques such as seed priming, soil and foliar application, and growing plants in media rich in major nutrients (Semida et al., 2021). A number of factors should be taken into account in this context, such as identifying the most effective nanomaterials, (ii) determining the optimal dose without allowing any negative effect on growth and physiology of plants, and (iii) analyzing the impact of nanoparticle on absorption, accumulation, and transport of other mineral elements. Zinc is one of the essential micronutrients in the development of plant as it is the cofactor for many important enzyme within the plant system. A worldwide shortage of zinc is a major cause for concern. Agricultural output in West Asia has taken a serious hit in recent years due to climate change impacts like severe droughts, the spread of salinized land, and dust storms. Many studies have shown that giving plants extra zinc can help them deal with the stresses they face from the outside environment (Hussein & Abou-Baker, 2018). When zinc is applied from outside either as foliar spray on leaves or as fertilizer on the soil, the bioavailability of Zn in the edible plant part also enhances. Zinc enrichment through foliar fertilization (Fig. 6.1) is by far was found to be an effective and environment friendly method to boost crop yields. Substances applied to leaves can enter the plant either through the cuticle or the stomatal pathway. The Zn content of rice can be greatly increased by applying zinc foliar fertilizer during the flowering stage (Alloway, 2008). However, the foliar spray is not successful every time. Due to raining the foliar spray may wash off the leaves and get leached into the soil. Thus, reducing the effectiveness of foliar application (Loneragan & Webb, 1993). Adding zinc fertilizer to the soil improves crop yields and boosts the Zn content of harvested grains. Zn fortification of rice has been the subject of numerous studies, all of which have found that adding zinc to the soil improves crop yield when used in conjunction with other fertilizers (Loneragan & Webb, 1993). Unfortunately, Zn fertilizer is less effective in soil because iron and aluminum oxides, clay minerals, and humus can adsorb and fix Zn ions (Yang et al., 2007). In addition, unutilized Zn fertilizer will build up in farmland soil, which may harm agricultural ecosystems. As a result, it is important to look for a new Zn-containing fertilizer that can replace traditional Zn fertilizers and has high positive performance with low negative environmental impacts (Du et al., 2019).

Table 6.1 Extensive information on the various published studies involving the biofortification applications of ZnO-NPs

Plants	Scientific name	Concentration of ZnO-NPs	Preparation method of ZnO-NPs	Characterization of ZnO-NPs	Application methods	Main finding	References
Common bean	*Phaseolus vulgaris*	10–40 mg/L	Chemical	20 nm	Foliar spraying	The highest seed yield was achieved with ZnO-NPs at a concentration of 30 ppm	Salama et al. (2019)
Wheat	*Triticum aestivum* L.	20–1000 mg/L	Chemical	˂100 nm	In soil	In comparison to ZnSO4, ZnO-NPs increased Zn in grain while having no effect on Zn in leaf	Du et al. (2019)
Wheat	*Triticum aestivum* L.	25–100 mg/L	Chemical	20–30 nm	Seed primming	ZnO-NPs increased photosynthetic pigments and crop yield	Rizwan et al. (2019)
Soybean	*Glycine max* L.	2 mg/kg	Chemical	18 nm	Pot culture	ZnO-NPs increased grain yield and nutrient utilization by regulating nutrient availability and boosting P uptake by 14%	Dimkpa et al. (2019a)
Sorghum	*Sorghum bicolor* L.	1, 3, and 5 mg/L	Chemical	18 nm	Pot culture	ZnO-NPs increased grain nitrogen, potassium, and zinc contents under drought stress help in biofortification under abiotic stress	Dimkpa et al. (2019b)
Eggplant	*Solanum melongena* L.	50–100 mg/kg	Chemical	<200 nm	Foliar spraying	ZnO-NPs increased crop production under drought	Semida et al. (2021)
Sesame	*Sesamum indicium* L.	3–10 mg/L	Biogenic	10 nm	Pot culture	The germination and growth of sesame plants have been boosted by biogenic ZnO-NPs	Umavathi et al. (2021)
Loquat	*Eriobotrya japonica*	12 mg/mL	Biogenic	<50 nm	Petri plate culture	Antimicrobial activity with fortification use	Shabaani et al. (2020)
Wheat	*Triticum aestivum* L.	40, 80, and 120 ppm	Biogenic	<50 nm	Soil culture	At a dose of 80 ppm, ZnO-NPs caused the greatest increases in height, seed weight, yield, and biomass	Sheoran et al. (2021)

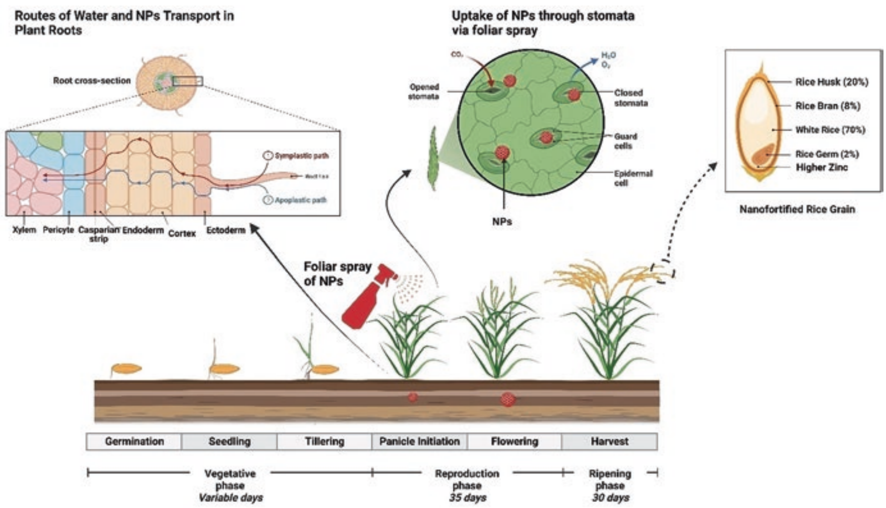

Fig. 6.1 Diagrammatic representation of mechanism of uptake of ZnO-NP-based fertilizer by rice plants through foliar spray or applying in soil that uptake by root via symplastic or apoplastic pathways and stomata that increased Zn contents into rice grain

6.2 Soil Zinc Availability

There are five different forms of zinc that plants can absorb: (a) free and complexed ions in soil solution; (b) nonspecifically adsorbed cation; (c) ion occluded mainly in soil carbonates and Al oxide; (d) biological residues and living organisms; and (e) lattice structures of primary and secondary minerals (Singh, 2008). Roots primarily uptake zinc as divalent cations (Zn^{2+}). Root uptake of organic ligand-Zn complexes has been reported, however (Tapiero & Tew, 2003). Two mechanisms occur in plant when uptaking the mineral in the form of divalent cations just like Zn^{2+} by plants, each of which is dependent on a different ligand secreted by the roots (Yang et al., 1994). The first strategy in which the expulsion of reductants, organic acids, and H+ ions takes place may enhance the Zn complexes solubility (Zn phosphates, hydroxides, etc.) and release Zn^{2+} ions for absorption by root epidermal cells (Bialczyk & Lechowski, 2008) (Fig. 6.1). Root epidermal cells can also receive Zn via the influx of phytosiderophores, which first form stable complexes with the metal. This mechanism, however, is specific to the roots of cereal crops. In order to chelate and absorb metals, plants use phytosiderophores, which are organic compounds with a low molecular weight (Cakmak et al., 2008; Hershfinkel et al., 2007).

When compared to the average total concentrations of around 64 mg Zn kg^{-1}, Zn concentrations in the soil solution are typically low (4–270 lg l^{-1}). Solubility is found to have a strong negative correlation with pH of the soil, with concentrations as high as 7137 lg l^{-1} being found in extremely acidic soils (Kabata-Pendias, 2010). The "total" Zn content, pH and redox conditions, calcite ($CaCO_3$) and organic matter contents, concentrations of all ligands capable of forming organo-Zn complexes, rhizosphere microbial activity, presence of other micro- and macro-elements (especially P) and their concentrations, and moisture content of the soil are the major factors controlling the amount of Zn which is in available form to the plants. The occurrence of Zn in soil is also affected by high $CaCO_3$ contents, relatively high organic matter contents (3%), neutral or alkaline pH, high available P status, high bicarbonate (HCO_3^-) and/or magnesium (Mg) concentrations in soils or irrigation water (particularly in paddy soils), prolonged waterlogging (e.g., paddy soils), and high sodium (Na), calcium (Ca), and potassium (K) concentrations in soils are all associated with the occurrence of zinc (Alloway, 2008; Kabata-Pendias, 2000).

6.3 Agricultural Crop Zinc Deficiency

The initial cases of acute zinc deficiency in the crops have been reported in literature in 1937. However, the first case of Zn deficiency in human was reported in the 1960s (Martens & Westermann, 2018). A big section of world has experienced acute Zn deficiency after the green revolution as a result of introduction of new high-yielding varieties within system over the past 5 decades. These new crop varieties often had lower yields and were Zn inefficient compared to older, locally adapted (landrace) varieties. In order to show their maximum yield potential, these varieties require more nitrogen (N), phosphate (P), and potassium (K) fertilizers, and in some cases, the soil's pH must be raised through liming (Graham & Rengel, 1993). In general, problems caused by Zn deficiency were made worse by high concentrations of P and high pH level. These varieties, however, are no longer high yielding. Rather they are now have yield plateau in the areas where they were introduced to serve the purpose of high yield and not just in crops showing clear deficiency symptoms (Graham & Rengel, 1993). This was later found to be hidden Zn deficiency. For instance, beginning in the 1960s, Turkey was able to increase its annual wheat grain production by ten million metric tons (Mt) while using the same amount of arable land. This was made possible by the introduction of new wheat varieties and an increase in the intensity with which wheat was grown. However, Zn deficiency became increasingly common in some regions, such as Central Anatolia, and was ultimately identified as the cause of poor yields by a NATO-funded research programs in the 1990s. With the application of Zn fertilizer, wheat yields in an area where they had been as low as 0.25 t ha^{-1} were increased by a factor of 6–8. As a whole, adding 10 kilograms of zinc per hectare increased the yield of bread wheat (*Triticum aestivum* L.) by 43% (range, 5–550%) (Bowen, 1986).

6.4 Physio-Biochemical and Molecular Importance of Zn

Zinc influences glycogen synthesis and gluconeogenesis, two pathways involved in carbohydrate metabolism (Zlobin, 2021). In most cases, zinc has no effect on plant respiration. Depending on the plant species and the severity of the deficiency, a zinc deficiency can cause a reduction in net photosynthesis of 50–70%. Carbonic anhydrase enzyme activity may be down, which may contribute to less efficient photosynthesis (Tu et al., 2012). Dicotyledon carbonic anhydrase is a larger molecule and contains more zinc than monocotyledon carbonic anhydrase (such as the cereals). Carbonic anhydrase activity in C3 plants is not directly correlated with photosynthetic carbon dioxide assimilation or plant growth when zinc availability is varied (Tu et al., 2012). Other enzymes in photosynthesis rely on zinc, such as ribulose 1,5-biphosphate carboxylase (RuBPC), which is present in navy beans, barley, rice, and pearl millet and is responsible for catalyzing the first step in photosynthesis, carbon dioxide fixation (McCall et al., 2000).

Due to reduction in starch content, enzyme activity, and the number of starch grains in zinc-deficient plants, it is possible that zinc acts as a major player in starch metabolism (Choukri et al., 2022). Sugar and starch levels in cabbage leaves were found to be higher in zinc-deficient plants, while bean root carbohydrate levels were found to be lower (Zhang et al., 2014). Although the precise cause of the sucrose transport impairment is unknown, it may be related to zinc's function in maintaining the stability of biomembranes (Faisal et al., 2021). The concentration of free amino acids was 6.5 times higher in zinc-deficient bean leaves than in control, but it decreased after administration of zinc for 48 or 72 h, and the protein content increased (Zhang & Monteiro-Riviere, 2009). Zinc is required for the enzyme RNA polymerase to function, and it also shields the ribosomal RNA from degradation by ribonuclease (Thakur et al., 2018). Since zinc is essential for protein synthesis, meristematic tissue, which is where a lot of cell division and nucleic acid and protein synthesis takes place, must have relatively high-zinc concentrations. Zinc's role in maintaining the integrity and activity of DNA has the most fundamental impact on protein metabolism (Chandrasegaran & Carroll, 2016).

6.5 Role of ZnO-NPs in Nano-Fortification

Use of Zn nanoparticle as the way to improve the plant uptake is very recent approach. The effectiveness of Zn fertilizers is also regulated by particle size. With the decreasing size of particle, the number of particles per unit weight increases along with its surface area which in turn enhances the dissolution of fertilizers with low water solubility, such as zinc oxide (ZnO). Zinc nanoparticle application has been proven to be a successful method of increasing the zinc content of crops by the increased zinc content of shoots in response to ZnO-NPs supplementation. Granular

Zn sulphate ($ZnSO_4$) (1.4–2 mm) was slightly less effective than fine $ZnSO_4$ (0.8–1.2 mm), whereas granular ZnO was completely ineffective. Reduced granule size was associated with a gradual increase in Zn uptake. Smaller granules were used for the same weight because 1.5-mm granules weigh less than 2.0- or 2.5-mmgranules, thus allowing better distribution and uptake of Zn, which is due to higher surface area of Zn fertilizer. To increase the effectiveness of fertilizers for better uptake and higher yields, a lot of work has been done, with a focus on particle size. The root development of *Cicer arietinum* was aided by the use of ZnO-NPs. ZnO-NPs have been seen to affect *Cicer arietinum* seed germination and root development. Some following points may be considered while using ZnO-NPs as nano-nutrients:

- Nano-dosing: Where the application of significantly high dose to the plants may be toxic for the physiology and functioning of plant system and, by extension, for humans who consume these plants. As a result, biofortification can't begin until the optimal nutrient dose has been determined.
- Preparation and application method of nutrient: In the problematic soils like saline soil, alkaline soil, soil with low or high pH, and others, the application of nutrient directly on plants through foliar application nano-nutrient is an effective option. Nutrients that are made through biological process, in particular, are preferred due to their low toxicity and environmental friendliness.
- Biofortification techniques, such as seed priming with engineered nanomaterials, are being studied as a potential way to combat malnutrition. Nutrient enhancement through biofortification can be achieved through several ways, like soil enrichment with candidate nutrient-containing fertilizer, foliar spray, and seed priming with the candidate nutrient.

6.6 Conclusion

Zn being a micronutrient is required to plant as human body in a small amount. However this trace amount have major role in proper functioning of plant and human system as Zn is the cofactor for many enzymes. As the available concentration of Zn is low in the grain, it poses the negative impact on the yield potential, thus impacting the food security. To overcome this, whenever the crops are treated with the correct amount of ZnO-NP fertilizer with careful application timings, the Zn concentrations within the increase efficiently. To ensure that everyone has access to zinc-enriched diets, biofortification of grains and other crops like pulses is a great idea for the people around the globe, more specifically for the rural population. The nano-biofortification strategy has potential as a tool for combating malnutrition. This method, especially in its biological nano-form, shares some of the benefits of nano-fertilizers, such as efficiency and reduced usage. This method is still in its early stages and needs more testing before it can be implemented on a global scale.

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Chapter 7
Economic Valuation of Ecosystem Services

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7.1 Introduction

An ecosystem is defined as the recycling of nutrient streams along paths that consist of living subsystems, connecting living and nonliving subsystems (Shaw & Allen, 2018). In other words, an ecosystem is a complex and dynamic combination of all plant communities, animals, and living microorganisms and interactions with components and the environment (Cowan, 2007). Ecosystems provide several goods (e.g., food production) and services (e.g., air regulation) to humans and contribute to their well-being and survival (Englund et al., 2017). These services are called ecosystem services (ESs). ESs or the benefits that people receive from ecosystems (MEA, 2005) refer to the services and products provided by an ecosystem under appropriate ecological conditions (Xie et al., 2021). The concept of ES introduces the idea that human societies are closely dependent on natural ecosystems and the organisms that host them (Barot et al., 2017). So, these services are essential for human livelihood, well-being, and health (Balasubramania, 2020; Li et al., 2020; Sannigrahi et al., 2021). According to the importance of ES in human life, the capacity to provide ecosystem services has changed significantly due to changes in human activities and the natural environment, and as a result, the supply of ES cannot sate the demand (Han et al., 2022). Many studies show decrease in ES in wetlands, forests, grasslands, and natural habitats (Zarandian et al., 2017; Xie et al., 2018; González-García et al., 2020; Chen et al., 2021; Rötzer et al., 2021; Xu et al., 2021; Sheng et al., 2022); ignoring the value of ES in these ecosystems can reduce the protection of these ecosystems (Sarkheil et al., 2021). The loss of ES has prompted many prominent researchers and organizations around the world to come up with new proposals to re-evaluate the relationship between community and ESs (Costanza et al., 2014). One of the mechanisms that can be used to anew this relationship is to determine the economic values (Perez-Verdin et al., 2016). Economic valuation, in addition to facilitating the decision-making process, provides essential information for the better management of ecosystems and their suitable consumption (Badamfirooz et al., 2021). Accordingly, in order to better understand the value of ES, it is necessary to quantify them and then economically evaluate nonmarket resources in order to identify all the resources available in a community. Although the economic valuation of ES is one of the main tools of environmental protection (Balasubramania, 2020), but the application of these methods in real policy is still a rare phenomenon (Merriman & Murata, 2016).

In this regard, the aim of this study is to review the concepts of economic valuation of ES as an important tool to increase attention to ES in environmental decisions and planning.

7.2 Theoretical Foundations

7.2.1 The Importance of ES for the Economic and Social Well-Being of the People

Ecosystems, with the goods and services they provide, underlie all aspects of human, cultural, social, and economic well-being (Wood et al., 2018). Unfortunately, currently, ecosystems around the world are being destroyed by the pressure of human intervention, which has serious consequences for nature's ability to provide ecosystem goods and services (EGS) (MEA, 2005). The main reason for the decline of many EGS is to ignore their value and importance (Wittmer & Gundimeda, 2012). Meanwhile, given that changes in biological or physical parameters are more meaningful to humans, ESs were used to link ecological or biophysical changes to economic and social consequences (Sharon et al., 2018). In this regard, given the importance of ES, many efforts have been made to identify methods in which ecosystems are useful to humans and the feedback between management actions and their impact on ES (Wood et al., 2018). Ecosystem goods are products or outputs of nature that are extracted and consumed by people. Also, ES including conditions, processes, and functions of natural ecosystems and related species that provide sustainability and the needs of human life (De Groot et al., 2002). These services are vital inputs for the production of economic goods that are necessary for the sustainability of life support systems and create a wide range of nonmarket benefits and very high economic value (Heal, 2000).

Generally, the role of ecosystem services in the economic and social welfare of people is due to the following two main reasons:

- Natural resources are inputs for the production of goods.
- They lead to the preservation of natural assets (an asset is defined as something that has value or benefits, and natural assets also refer to the inventory of natural resources such as pastures, forests, Water assets, and geological), which is done for two reasons: first, by reviving and reproducing the capacities of natural assets and second, by absorbing by-products from the production process.

7.3 Classification of ES

Many concepts of ES have led to the emergence of various related classifications. Classification systems are hierarchical approaches to organizing information so that data can be easily comparable (US Bureau of Labor Statistics, 2019). These systems also have a flexible thesaurus, vocabulary, and structure that balance stability with the needs of new research (Finisdore et al., 2020). The most important of these classifications are described by Costanza et al. (1997) in the *System of Environmental-Economic Accounting Central Framework* (SEEA, 2003), Millennium Ecosystem

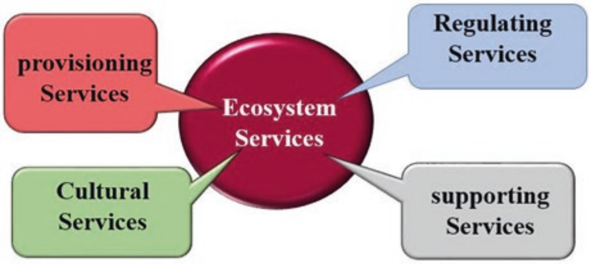

Fig. 7.1 The types of ES

Assessment (MEA) (Boyd & Banzhaf, 2007; Costanza, 2008; Daily et al., 2009; De Groot et al., 2010), the Economics of Ecosystems and Biodiversity (TEEB, 2010) (Staub et al., 2011), and the Common International Classification of ES (CICES, 2013) (FEGS-CS, 2013; NESCS, 2015). The variety of classification methods suggests that depending on the objectives and understanding of the observer, there are many ways to classify ES. However, from a system's point of view, the selected set of services should include a comprehensive analysis. Currently, most classifications are based on MEA ideas (Sumarga et al., 2015; Tekken et al., 2017; Carrilho & de Almeida Sinisgalli, 2018). The advantage of defining and classifying MEA is its simplicity. According to the MEA, these services include four categories (Fig. 7.1).

The MEA simply defined these services:

- Provisioning: Goods taken from ecosystems
- Regulating: Benefits of regulating ecosystem processes
- Cultural: Nonmaterial benefits of ecosystems
- Supporting services required to produce other ESs

The first three services directly affect humans, and the fourth service is critical to the continued provision of other services by ecosystems (MEA, 2005). The types of ES based on the MEA are shown in Table 7.1.

7.4 Economic Valuation of Ecosystem Services (ESV)

Monetary arguments for recognizing the relative importance of different forms of ESs and natural capital may not be universally accepted, but it can be useful and convincing for decision-makers. In other words, if the benefits provided by nature are not valued, they are considered "worthless," and the current trend of decay and destruction of natural systems would be continued (Mohammadyari & Zarandian, 2022). According to this issue, the use of economic valuation has many benefits to highlight the significance of ecosystems (Costanza et al., 2014). Evaluating ES is a tool to express the relative importance of the benefits that ecosystems provide to people. ESV was first used in the early 1990s (Liu et al., 2010), and it was approved at the Conference (COP) in Nagoya in 2010. In this report, the value of economic evaluation is mentioned as a key tool to better understand the mainstream of

Table 7.1 ES classification

Category	Subcategory	Example/definition
Provisioning services	Genetic resources	Genes used to increase crop resistance
	Raw materials	Fiber (timber, wood, fertilizer, and fodder)
	Biochemicals	Ginseng, garlic, and plant extracts for pest control
	Ornamental resources	Decorative plants and artisan work
	Freshwater	Groundwater, rainwater, and surface water
	Food	Products (cereals, vegetable, and fruits)
		Livestock (chicken, cattle, and other livestock)
		All kinds of fish and shrimp
		Wild foods (mushrooms, fruits, and nuts)
Regulating services	Climate regulation	The effects that ecosystems have on the global climate through the removal of greenhouse gases or aerosols
	Air quality regulation	The effects that ecosystems have on air quality by emitting chemicals into the atmosphere (as a source) or by removing them from the Earth's atmosphere (as a sink)
	Water regulation	The effect of ecosystems on the timing and the number of watercourses, floods, and watershed recharging, especially in terms of water storage potential in the landscape
	Erosion control	The role that vegetation plays in soil stabilization
	Regulation of diseases	The role of ecosystems in the prevalence or abundance of human pathogens
	Pollination	Forest bees help pollinate plants
	Organizing natural disasters	Capacity of ecosystems in reducing disasters caused by natural disasters such as storms and tsunamis
Cultural services	Ethical values	Inspirational, religious, aesthetic, and intrinsic values
	Existential values	Belief that all species, regardless of their usefulness for humans, have protective value
	Recreation and ecotourism	Walking and cycling
Supporting services	Nutrient cycle	Processes by which nutrients such as phosphorus, sulfur, and nitrogen are extracted from mineral, aquatic, and atmospheric sources, or eventually returned to the atmosphere, soil, and water as a cycle of living organisms
	Soil formation	The process of decomposition of organic matter to form soil
	Photosynthesis	Production of living materials through the absorption and accumulation of energy by living organisms
	Water cycle	Flow of water through ecosystems in solid, liquid, and gas forms

biodiversity. Subsequently, ESV studies increased rapidly. In classical economics, the share of nature services is related to the value of their use (Häyhä & Franzese, 2014). While neoclassical welfare economics defines the economic value of goods or services as a measure of well-being (in monetary units) after production and consumption (Burkhard & Maes, 2017). Thus, according to the definition of neoclassical economics, economic value arises from the mental preferences of individuals (Häyhä & Franzese, 2014). Acceptance of individuals to compensate for the loss of an environmental benefit or increase of an environmental loss is called willingness to pay (WTP) or willingness to accept (WTA) (Mohammadyari et al., 2019). Monetary valuation of ecosystems provides valuable information about social benefits and costs to policymakers and environmental managers. So the estimation of monetary value for ESs has become a tool to increase the importance of these services in the decision-making process (Schild et al., 2018). ESV, in addition to increasing the motivation to protect the ecosystem in the public and private sectors, also helps reduce poverty in developing countries (Christie et al., 2012). In fact, economic assessment helps to manage ecosystem-based management, and tools are important for supporting ecosystem management. For this purpose, market price or quasi-market price is used to estimate the social and economic benefits of ES (Folkersen, 2018). Using a nonmarket approach is for goods such as water quality and the like for which there is no specific market. In a real market, the economic value of goods or services is determined by their supply and demand. Supply of goods or services refers to the cost of production for producers in order to provide a good or service. On the other hand, the benefit or welfare that consumers gain from a good or service is called demand (Burkhard & Maes, 2017). Many studies have examined ESV (O'garra, 2012; Vo et al., 2012; Martín-López et al., 2014; Cuni-Sanchez et al., 2016; Jiang et al., 2017; Rewitzer et al., 2017), and a review of the ESV literature emphasizes that the need for ES is not only due to the direct goods and services they offer, but also noncommercial services such as recreational and aesthetic aspects play an important role in human mental and physical health, and this indicates the high value of nonmarket services of natural resources in comparison with their goods and market services (Morsali et al., 2020). ESV can serve a number of purposes, including the following:

- Communicating the value of ES by highlighting their economic contributions to societal goals.
- Comparing the cost-effectiveness of an investment.
- Evaluating the impacts of development policies. This could include evaluating the ES costs associated with habitat conversion, runoff, or pollutant discharge. It could also include looking at the benefits of increased investment in enforcing environmental regulation and in strengthening resource management.
- Building markets for ES.

Natural resource economists have considered the economic welfare benefits of nature for decades. They use a framework of total economic value to reflect the multiple different types of values that ecosystems can provide (Fig. 7.2). This framework includes both use and nonuse values that individuals and communities

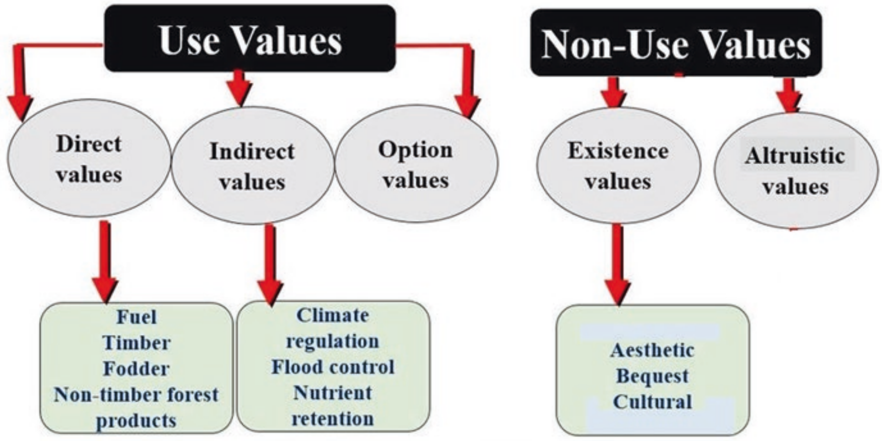

Fig. 7.2 Total economic value

gain or lose from marginal changes in ES. Use values arise from consumption, while nonuse values require the use or consumption of the ES. Use values can easily be measured by market prices or other tools and involved in decision-making processes. There is a good consensus among environmental economists that in addition to use values, natural resources may have values that are unrelated to actual direct or indirect use. These values, known as nonuse values, do not involve any observable behavior and are only the result of a simple mental experience. Therefore, nonuse values can be observed in market purchases or based on functions, nonconsumption, or intrinsic values in goods can be deduced inseparably (Mohammadyari & Zarandian, 2022).

As shown in Fig. 7.2, use values are divided into the following three categories:

- *Direct-use value:* The value of all goods and services resulting from the direct or planned use of ecosystems, the consumption of resources (such as fodder and food), or the nonconsumption of services (such as regulation). They usually include production services.
- *Indirect-use value:* These categories include regulating and support services and are derived from the performance of ecosystems underlying direct-use activities.
- *Option value:* The value that a person places on having the authority to use a service or resource directly or indirectly in the future, even if it is not currently used.

  Nonuse value or passive value refers to the knowledge of ecosystem conservation and includes all services (such as provisioning, regulating, cultural, and supporting). In fact, this value does not include the actual use of ecosystem goods and services. According to Fig. 7.2, nonvalue uses fall into four main categories:
- *Existence value:* A value that depends on knowledge about the existence of species and ES is called existential value. Some studies consider the bequest value as part of existence value, while others place it in a separate category. The value

of bequest refers to the value that human beings place on the availability of goods and services for the future.

- *Altruistic value:* The value that people place on the availability of ecosystem resources or services to others in the current generation is called altruistic value.

In order to evaluate ES, three basic approaches are considered by total economic value (TEV).

1. In market transactions that are directly related to ES, values are obtained.
2. In parallel market transactions that are indirectly related to the intended ES, values are extracted.
3. Using the creation of hypothetical markets, ES value information is evaluated (Croci et al., 2021).

7.5 ES Economic Valuation Methods

Economic valuation of all the benefits that humans derive from ecosystems is practically impossible. Because ecosystems have spiritual, religious, and historical values, for most people the valuation of these values in utilitarian ways is not fully understood. Accordingly, public opinion is questioned for the value of such services. Given that almost 80% of ES are not traded in the markets, in this regard, the estimation of their economic value depends on different methods of economic valuation (Carrilho & de Almeida Sinisgalli, 2018). Therefore, ESV methods have always been considered by experts as a central issue in environmental economics and natural resources and have also been criticized by a wide range of environmentalists and natural resources advocates alike. Failure to properly calculate the value of some environmental resources has consequences that have negative effects on the environment, ecosystem, and society. ESV includes a wide range of methods that can be implemented in a variety of ways and in combination with other techniques. Several methods have been proposed for the economic valuation of ecosystem goods and services. The design of these methods has been based on understanding the complexity of the natural environment using economic analysis (Burkhard & Maes, 2017). Market price, contingent valuation (CV), choice experiment (CE), travel cost (TC), benefit transfer (BT), contingent behavior (CB), replacement cost (RC), damage cost, net present value, and hedonic pricing (HP) are the most important methods. At present, the efficiency of these methods is well-established and confirmed. The choice of valuation methods depends on many factors such as the type of service, the purpose of the study, the time, and the availability of resources (Dang et al., 2021). Table 7.2 presents a fairly comprehensive overview of the methods used by researchers all over the world for a variety of ES.

According to the literature (Table 7.2), most of the methods that researchers have chosen to study ESV are CV and market price methods, respectively. According to the classification of monetary valuation methodology, the CV is in the category of

Table 7.2 A review of studies on the economic valuation of ecosystem services

Category	Valuation method							Subject	References
	CV	CE	TC	BT	CV and TC	Market price	CB		
Provisioning services	*							Wood	Groot et al. (2002)
	*							Mining Ecosystem restoration	Beltrán Morales et al. (2005)
	*							Drinking water	Avilés-Polanco et al. (2010)
						*		Food	O'garra (2012)
		*						Food	Camarena et al. (2012)
	*							Drinking water	Almendarez-Hernandez et al. (2013)
	*							Drinking water	González-Davila (2013)
						*		Food	Martín-López et al. (2014)
						*		Food and fiber and fresh water	Považan et al. (2015)
	*							Irrigation water	Vélez-Rodríguez et al. (2015)
	*							Food	Romano et al. (2016)
	*							Drinking water	Peng and Oleson (2017)
	*							Food	Torres-Miralles et al. (2017)
						*		Food and fiber and fresh water	Schirpke et al. (2017)
						*		Food	Carrilhoand de Almeida Sinisgalli (2018)
						*		Food and fiber	Marta-Pedroso et al. (2018)
	*							Drinking water	Mohammadyari et al. (2018)
						*		Food and fiber	Ramel (2020)

(continued)

Table 7.2 (continued)

Category	Valuation method							Subject	References
	CV	CE	TC	BT	CV and TC	Market price	CB		
Regulating services	*							Clean air, water conservation	Larqué-Saavedra et al. (2004)
	*							Watershed protection	López Paniagua et al. (2007)
	*							River restoration	Ojeda et al. (2008)
	*							Watershed protection	Silva-Flores et al. (2010)
	*							Watershed protection	del Ángel Pérez et al. (2011)
	*							Watershed protection	Sánchez Brito et al. (2012)
		*						Carbon sequestration	Balderas Torres et al. (2013)
	*							River restoration	Jaramillo-Villanueva et al. (2013)
		*						Coastal wetlands	Camacho-Valdez et al. (2013)
								River drainage	HE et al. (2015)
				*				Climate and erosion and water	Považan et al. (2015)
	*							Water shortage	Bozorg-Haddad et al. (2016)
	*							Air and erosion and water	Torres-Miralles et al. (2017)
						*		Climate	Schirpke et al. (2017)
				*				Cyclones	Vink and Ahsan (2018)
				*				Climate and erosion	Marta-Pedroso et al. (2018)
	*							Polluted urban lake	Sebo et al. (2019)
						*		Climate	Ramel (2020)

Cultural services	*							Marine resources	
		*						Marine recreation	Wielgus et al. (2009)
							*	Tourism	Kragt et al. (2009)
	*							Coral reef protection	
							*	Tourism	Prayaga et al. (2010)
	*							Recreational	Moradi et al. (2011)
	*							Urban trees	Lo and Jim (2015)
			*			*		Recreation and ecotourism	Považan et al. (2015)
					*			Lake	Jala and Nandagiri (2015)
	*					*		Recreation and ecotourism	Gandarillas et al. (2016)
	*							Natural area	Almendarez-Hernández et al. (2016)
	*							Historic sites	Kuhfuss et al. (2016)
	*							Coral reef management	Trujillo et al. (2016)
				*	*			Recreation and ecotourism	Ninan and Kontoleon (2016)
	*							Forest park	Mohammadi Limaei et al. (2016)
		*						Agricultural heritage and aesthetics	Rewitzer et al. (2017)
	*							Recreation and ecotourism and aesthetic	Torres-Miralles et al. (2017)
			*					Recreation and ecotourism	Schirpke et al. (2017)
			*					Geopark	Pourbalighy and Hejazi (2018)
		*						Recreation and ecotourism	
	*							Spiritual and religious	
			*					Local entertainment	Kipperberg et al. (2019)
				*		*		Recreation and ecotourism	Ramel (2020)
			*					Recreational wetland	Morsali et al. (2020)
	*							Tourism and aesthetic	Amirnejad et al. (2020)
						*		Aesthetic	Hatan et al. (2021)

(continued)

Table 7.2 (continued)

Category	Valuation method							Subject	References
	CV	CE	TC	BT	CV and TC	Market price	CB		
Supporting services	*							Biodiversity	Bräuer (2003)
		*						Biodiversity	Juutinen et al. (2011)
	*							Biodiversity	Kamri (2013)
				*				Biodiversity	Gandarillas et al. (2016)
	*							Biodiversity	Ninan and Kontoleon (2016)
	*							Biodiversity	Parsons and Myers (2017)
	*							Biodiversity	Torres-Miralles et al. (2017)
	*							Biodiversity	Ferreira et al. (2017)
	*							Biodiversity	Marta-Pedroso et al. (2018)
	*							Biodiversity	Molina et al. (2019)

Various services		*						Wetland	Birol et al. (2006)
	*							Forest	Tao et al. (2012)
	*							National park	Kamri (2013)
					*			Lake	Jala and Nandagiri (2015)
		*						Forest	Balderas-Torres et al. (2015)
					*			Cultivated land	Huang and Wang (2015) and Považan et al. (2015)
	*							Green space	Song et al. (2015)
	*							Wetland	Siew et al. (2015)
	*							Urban park	Latinopoulos et al. (2016)
	*							Coastal promenade	Lee and Yoo (2016)
	*							Beaches	Peng et al. (2017)
		*						Natural functions of lake	Vakili Ghaserian et al. (2017)
			*					Areas of high natural value	Gawrońska et al. (2018)
				*				Beaches	Mehvar et al. (2018)
			*					Parks and protected areas	Jaung and Carrasco (2020)
	*							Provisioning and regulating in forests	Naime et al. (2020)
					*			Forest	Tahami Pour Zarandi and Bitars (2020)
				*				Wetlands	Badamfirooz et al. (2021)
	*							Coastal lagoon	
		*						Lake	
	*							Urban park	Silva et al. (2022)

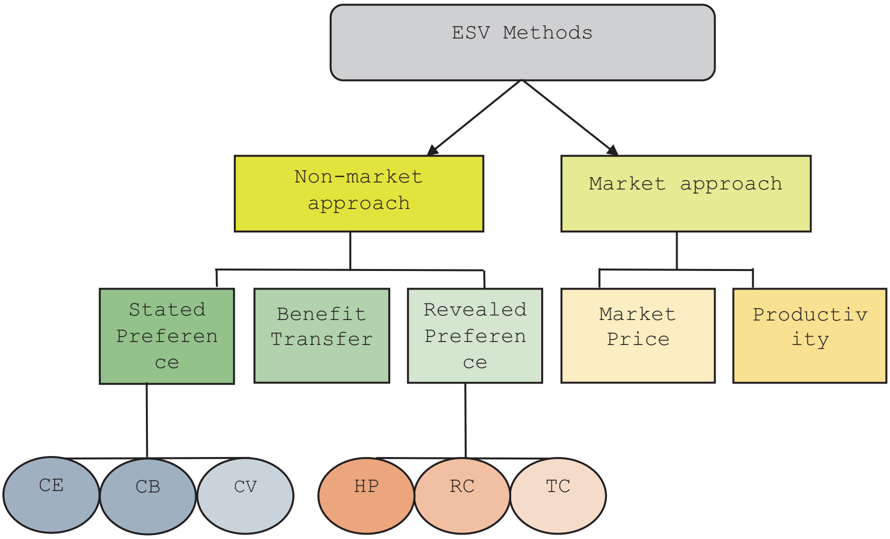

Fig. 7.3 Schematic view of ESV methods

nonmarket valuation, and the market price method is in the category of direct market valuation. In nonmarket valuation methods, the value of services and goods is formed through obvious preferred methods or hypothetical markets. In fact, in these methods, services and goods are not valued directly at market prices (Folkersen, 2018). In addition to the conditional valuation method, CB and CE methods are also in the category of nonmarket methods. Flexibility is the most important advantage of the CV method that is useful for all market and nonmarket goods and includes the types of benefits that humans derive from ecosystems, such as nonconsumption values and option values. However, the sensitivity of this method to conducting a survey is one of the disadvantages of this method, which limits the generalization of results. On the other hand, relatively easy and straight implementation is the strength of the market price method, but the main bug of this method is that if the market is distorted, prices do not show the true value of the service, which makes economic values biased (Naime et al., 2020). Figure 7.3 presents the methods of economic valuation of ecosystem goods and services.

The following are definitions of ESV methods.

7.5.1 *Market Approach*

Market-based methods fall into two categories, which are market prices and productivity methods.

7.5.1.1 Productivity Method

Using this method, the economic value of the benefits of the ecosystem used in the production chain for the commercial goods sold can be estimated economically. In this case, natural resources are considered part of production, so any change in their quality or quantity affects production costs and ultimately the price of the product (Badamfirooz et al., 2021).

7.5.1.2 Market Price Methods

In market price methods, the direct costs observed from the real markets related to their presentation as indicators are used to evaluate services or goods (Croci et al., 2021). In fact, this method is used to estimate the economic value of ecosystem goods traded in commercial markets. In this method, based on changes in a final product or service, the total economic surplus (producer and consumer) is estimated (Badamfirooz et al., 2021).

7.5.2 Nonmarket Approach

Nonmarket ecosystem services (such as aesthetics and tourism) are positive externalities that, if valued monetarily, can easily be used in economic decisions (Burkhard & Maes, 2017). Nonmarket methods include three categories: revealed preference, benefit transfer method, and stated preference method.

7.5.2.1 Revealed Preference

Revealed preference methods analyze the relationship between demands for certain market goods and the preferences of related nonmarket goods and services (Tinch et al., 2019). These methods are defined based on conventional and proxy markets and allow economists to use the actual choices of individuals in relevant markets to determine the value of environmental services. Thus, the value of nonmarket resources and public goods is obtained by using the consumption behavior of individuals in related markets (Mohammadyari & Zarandian, 2022). TC methods, RC methods, and HP are among the methods that fall into this category.

- TC Method

The TC method is estimated based on the time and cost of travel of people visiting a place, and the basic premise is that the costs for a person that incurs to visit a place of entertainment reflect the value of the person for that place. In this method, it is assumed that the value of the place or its recreational services reflects the willingness of people to pay to use that place. Zonal travel cost method (ZTCM), single and multiple site models, random utility approach, and individual travel cost method (ITCM) are different types of this method (Mohammadyari & Zarandian, 2022). Among these methods, two methods include ITCM and ZTCM are mostly used in the economic valuation of ES.

ITCM method: This method is based on a survey in which a questionnaire is asked for visitors in a place, questions about accommodation, number of visits and trips, expenses, etc. In the ITCM method, the costs that are spent on the consumption of facilities and recreational facilities of a particular place are presented as a symbol of price. These costs include travel expenses, entrance fees, location costs, and the amount spent on capital equipment (Flemming & Cook, 2008). This method is a suitable tool for economic evaluation of ES for the following reasons:

- This method is relatively inexpensive.
- It follows the conventional empirical methods used by economists to estimate economic values based on market prices.
- This method is based on the actual behavior of what people are actually doing, rather than their willingness to pay.
- The results obtained from this method are relatively easy to interpret and explain.

ZTCM method: This method is based on estimating the relationship between the number of people referring to a place and then the distance of their residential places from the desired place. The advantages of this method include the following (Fleming & Averil, 2008):

- ZTCM method is the only way to express the real reaction of the people about the facilities of the resort.
- The economic value of places and the comparison of their demand curves show the real reaction of the applicant to different places.
- The calculation method used in this method not only measures people's reaction to the existing supply according to economic and social factors but also by further studying the suggestions given by people; we can meet today's needs and predict the community in terms of facilities in different places, and thus, real information will be available to planners for future planning.

• RC Method

The RC method considers the cost incurred by replacing ES with artificial substitutes. This method is based on two main assumptions. First, the cost is not greater than the benefits of ES, and second, the secondary benefits of the replacement system are unrelated. Given these two assumptions, economic valuation is not exaggerated. An important advantage of this method is the ease of estimating cost information and saving time. On the other hand, the limitation of this approach is that alternative costs are not always a reliable measure of the benefits of ES, because artificial technologies do not usually produce all the services that an ecosystem provides. Accordingly, this method is more suitable for estimating the economic value of a single ES than multiple ES (Notaro & Paletto, 2012).

- HP Method

HP presumes that the price of a good contains the contributions from its several environmental characteristics and inherent (Xu et al., 2016). This method is more reflective of housing changes or land prices. So, using analytical techniques like multivariate regression, the WTP for each feature can be identified. This method is usually used to estimate the value of ES involved in providing welfare and facilities (Badamfirooz et al., 2021). It is recommended to use this method to estimate the benefits attributed to air pollution, water pollution, noise pollution, and access to urban green spaces.

7.5.2.2 Benefit Transfer Method

The benefit transfer method is based on the use of meta-analysis, which according to the results of a number of studies analyzes them in such a way that changes in the results found in those studies can be explained (Azis, 2021). So when there is not enough time to do economic valuation, this method would be suitable. This method uses the average standardized values of ES in each ecosystem, and for this purpose, it estimates the value of different ecosystem services using the Ecosystem Service Valuation Database (ESVD) (Badamfirooz et al., 2021).

7.5.2.3 Stated Preference Method

The basis of the methods of preferential techniques is to create a hypothetical situation for the respondents. This approach includes two methods, which are CV and CE:

- *CV Method*

CV is the most common way to estimate the amount of ES, which uses survey data to directly assess household preferences. In this way, by creating a potential market, respondents are asked to express their willingness to pay for services. This method is called conditional valuation because in this method; people are asked to express their willingness to pay based on description of environmental services and a specific hypothetical scenario. The basis of the method is to describe to the respondents the current state of a nonmarket commodity and how to improve it. They are also asked if they are willing to pay to improve the product (Perez-Verdin et al., 2016). The most important advantages of the CV method are flexibility, which is estimating the economic values of nonmarket interests and considering the values of use and nonuse (Mohammadyari et al., 2018). On the other hand, the subjectivity of the values reported by this method has been introduced as its main drawback (Krause et al., 2017). The CV method contributes to local policy planning by providing useful information on incentive-based opportunities for preserving natural environments by outlining how much individuals would be willing to pay for different aspects of environmental attributes. Nonetheless, the CV method fails to investigate how hypothetical changes in the quality of a good might affect the future demand of that good. This may be of greater relevance for developing countries whose economy is driven by a tourism industry based on vulnerable natural resources, such as rain forests (Folkersen, 2018).

- *CB Method*

Using the CB method, the revenue effect of a hypothetical future change on the quality of an ES can be estimated. In this regard, the respondents' WTP to visit a natural environment with its current environmental quality is compared with the WTP of individuals to return to the same natural environment in a hypothetical scenario (Folkersen, 2018).

- *CE Method*

The CE method is based on two theories of consumer theory and random utility. In order to economically assess environmental changes in the landscape, this method is usually used, which provides an opportunity to identify the values of changes in the characteristics of environmental goods. This is a benefit for landscape assessment because valuing changes in the valuation of a particular landscape as a whole is more difficult than the individual characteristics that describe a landscape. Eventually, in this method, it is possible to explore the WTP distributions for each feature (Rewitzer et al., 2017). In fact, in this method, which is based on a survey, participants are asked to choose their desired alternative from a set of options that are characterized by different levels of quantitative or qualitative characteristics. Usually the price of the product is one of these features. The great advantage of the CE method is that it not only estimates a value, but it is also able to rate, rank, or select an alternative that provides the greatest utility to the respondent. The main advantage of benefit transfer is to provide a relatively quick assessment of the economic value of ES.

7.6 Conclusions

In this chapter, first, the importance of ES in the welfare of the people are mentioned. Then, the importance of economic valuation of ESs is discussed, and finally, after reviewing the literature, the most widely used methods of economic valuation of ES are introduced. A comprehensive literature review is presented which can be useful for researchers in choosing the appropriate method for ESV studies and in addition can be considered as a guide for future research. As mentioned, ESV is a way to quantify the value of goods and ES, the main purpose of which is to highlight the importance of ES for human well-being and to inform decision-makers and planners in order to better manage system. Although ESV provides useful information for better ecosystem management, which in turn leads to human well-being, but economic valuation sometimes includes limitations which could be highlighted in two ways: First, the vulnerability of ecosystems has reached the threshold, and it is practically impossible to change them to the previous state, and second, in the case of reversibility, high costs are required. In such cases, economic valuation has a lot of uncertainty. On the other hand, monetary valuation can help to better calculate the costs of land degradation and the benefits of sustainable land management in decision-making. In terms of valuing various ES, international literature seems to have paid the most attention to cultural services. These services are more tangible and obvious than other services. On the other hand, the economic valuation of support services has received less attention than other services, which may indicate a research gap in this type of service. Furthermore, a review of the literature shows that in most valuation studies, the conditional valuation method has been considered and has a long history in ESV that has been able to provide reliable estimates for policymaking. Additionally, the MP method and CE method are the methods that have been used by researchers after the CV method, respectively. Two important advantages of the CV method, namely, ease of evaluation and its flexibility, have made researchers pay more attention to this method. In order to estimate the economic value of goods and services that are not directly defined by market prices, the use of this method is recommended. Because in this method, people express their desire to pay for goods and services in a simulated market. However, one of the main criticisms of this method is that sometimes the results are irrational and uncertain because respondents do not face real budget constraints and tend to say yes very easily. This uncertainty often leads to exaggeration, confusion, unreliability, and ultimately the production of useless information. One way to avoid uncertainty in this method is for respondents to be well-trained. To this end, the concept of value and the reason for the value of goods and services for human well-being must be clarified. In this case, the respondents with the previous background can answer the questions realistically, and so, obviously, in this case, the uncertainty is reduced. We recommend that in future studies the mechanisms of the CV method be reviewed to reduce the limitations of this method and to have more reliable results. Unlike the CV method, the CE method has greatly reduced concerns about uncertainty. Therefore, it can be a good alternative to the CV method. Although both CV and CE are theoretically grounded in the concept of stated preference methods, CE is generally considered to be a superior method. Therefore, if the conditions for the implementation of this method are provided, it is more favorable than the CV method. CE is free from the embedding effect, and different components of an attribute of interest can be experimentally evaluated in the same research setting. Although the purpose of economic valuation, regardless of how it is done, is to assist decision-makers in implementing public policy and environmental planning, but given the new approach to the test method, its optimal ability to evaluate ecosystem services has been confirmed. It is suggested that studies with this method be increased, and it is possible to use it as much as possible. Overall, due to the significant increase in pressure on natural ecosystems, ES valuation studies should be included in future projects. However, even with economic valuation studies, the full importance of ecosystems is not revealed to us because the economic importance of some ecosystems is still unknown. In this regard, ESV studies can help policymakers develop better strategies to identify essential ES for society, enhance general information on the importance of ES, and decrease the negative impact of parameters such as overgrazing and deforestation.

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Chapter 8
Malnutrition and Human Health: Causes, Consequences and Their Sustainable Remedies

Akanksha Soni and Irfan Khan

Contents

8.1 Introduction

Malnutrition is a common problem of the world since decades and refers to deficiencies, excesses, or imbalances in a person's intake of energy and/or nutrients. Malnutrition can be generally understood by two basic types, viz. undernutrition and overnutrition. Around the world, the frequency of undernutrition people is more

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in underdeveloped and developing countries such as African countries compared to the developed countries, such as European countries, the United States, etc.

The people living in low socioeconomic regions are more prone towards undernutrition problems and are facing worst due to high grade of food security issues. The problems of overnutrition can be more often observed in high socioeconomic regions that ultimately lead to overweight and obesity problems which intern increases the probability of causing a variety of noncommunicable diseases, viz. type 2 diabetes mellitus, polycystic ovarian syndrome (PCOS), cancer, cardiovascular diseases, etc. Malnutrition problems are prominent in case of infants, children, teenagers, adults and in aged persons. The malnutrition problem of children in society is seen as developmental impairment syndrome, resulting due to multiple factors (Beaton et al., 1990). The severity of child malnutrition is expressed medically in the form of marasmus and kwashiorkor.

The high prevalence of malnutrition is also a well-stablished fact in the case of hospitalized patients (Butterworth, 1974; Alberda et al., 2006; Marshall, 2018). A number of determinants are considered to assess the malnutrition status in patients such as disease burden, loss in muscle mass, weight loss, loss in appetite, etc. (Detsky et al., 1987; Ottery, 1996; Vellas et al., 1999; Cederholm et al., 2019).

In a study, it was found that the distribution of fat throughout the body has variable effects on human health, i.e. if there is high deposition at central abdomen, it reflects greater risk of cardiometabolic problems. Consequently, if the fat deposition is higher in the peripheral sites of the abdomen, it may be an indication of lower risk for cardiometabolic problems (Lotta et al., 2018). There may be several factors responsible for these problems, i.e. low per capita income, lack of employment opportunities, government policies and schemes at the national and global level, etc. Around 811 million people do not get food before bed every night, while around 135–345 million people are facing acute insecurity issues throughout the world since 2019 (WFP, 2022). It is also estimated that a total of 45 million people in 45 countries are on the verge of famine (WFP, 2022). The actual marker of noncommunicable diseases is the BMI (body mass index) among the population. A high BMI does not only reflect overweight and obesity conditions but the high prevalence of cardiovascular diseases (Wells, 2021). In a study, it was found that an increased prevalence of childhood BMI found greater as compared to the adults shows that there is higher probability of childhood obesity than adults (NCD-RisC, 2017) (Fig. 8.1).

It can be seen clearly that the prevalence of undernourishment in India is in the range of 5–14.9% during the year 2017–2019. While the lowest prevalence is depicted with light blue colour and recorded in the region of Australia, the United States,, Canada, Brazil, China, Russian Federation, Kazakhstan, etc. Consequently, the highest prevalence is shown with dark purple colour in the map and recorded as more than 35% in Madagascar (WFP, 2022). There may be variable determinants for the prevalence of undernutrition at different places. The poverty or unavailability of resources is the major cause of undernutrition. Poor sanitation and hygienic practices also result in malnutrition problems. In a study, it was found that poor oral

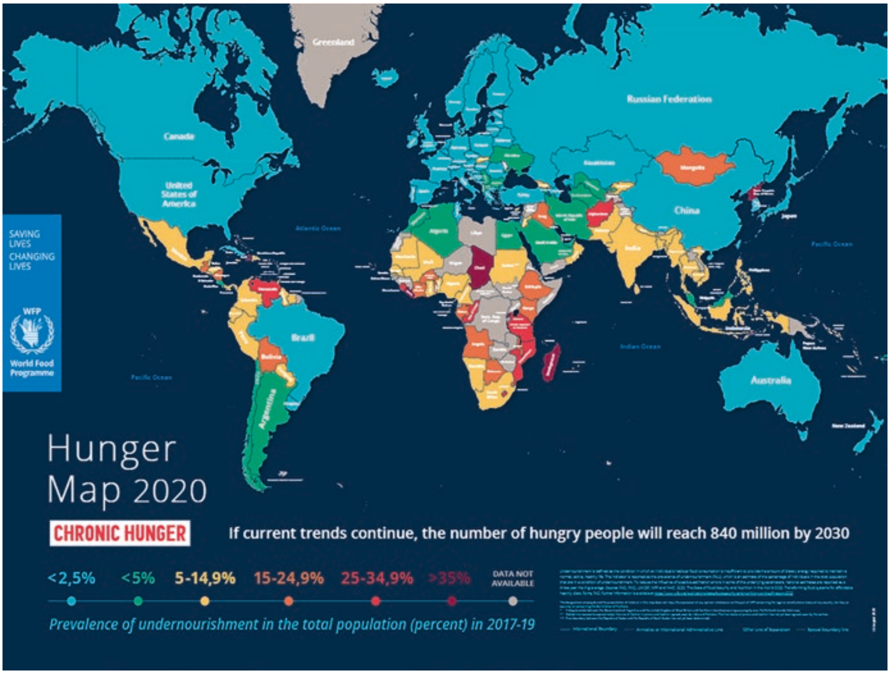

Fig. 8.1 Prevalence of undernutrition in the total population during 2017–2019. (Source: https://www.wfp.org)

health may be one of the determinants for malnutrition along with sarcopenia in aged persons (Azzolino et al., 2019).

As a general opinion, one of the major problems is the inequality of resources among the population. The efforts that can equalize the resources among the population could be employed to address food security issues. Many people around the world donate food as per their wish to their nearby community for a certain period of time. These things no doubt help the needy to get the foods and to get rid of food deficiency. But this is not a sustainable solution to alleviate the food security issue throughout the world.

In many religions, the act of donation is found very noble. As per Hindu mythology, the donation of food and water is known as an act of charity, and according to the Vedic scriptures, helping others is equivalent to helping Brahmins. In Hinduism, selfless acts of charity have been observed as karma yoga. So people following the Hinduism should follow whatever charity act is explained in their religion that would surely compensate to an extent to reduce malnutrition. Similarly in others, like in Christianity, it is believed that God's love and generosity towards humanity moves and inspires us to love and be generous in response. On the special occasion of Easter and Christmas, it is believed to make good foods, including meat dishes, and donate to the needy.

In Islam religion, donation (zakat) has been established as one of the five pillars of basic duties apart from profession of faith (shahada), prayer (namaz), fasting (roza) and visiting the holy place Mecca (Hajj). The result of the same can be observed in Muslim countries, where anybody can hardly find the problems of malnutrition, particularly undernutrition. In Islam, there is one festival termed Eid al-Adha; on this occasion, the sacrifice of authenticated animals is used to be done. It has been established a compulsory practice in Islam as mentioned in Qur'anic Surah Al Kawthar-108 which indicates the sacrifice in the name of Almighty God. Minimum one-third of the slaughtered animal must be donated to the needy or more as per the wish. This is the only day in a year when everybody get the food at least for a week. Similar activities have been performed by other non-governmental organisations in India and abroad where they distribute the foods to the needy, and this model has been proven a boon during covid-19 pandemic situation.

The outbreak of coronavirus has worsened food security problems throughout the world. To actually and honestly address the malnutrition problems and to devise a sustainable solution for the same, the scientific community, government and non-governmental organisations have to work in collaboration.

8.2 Malnutrition

The term 'malnutrition' has no universally accepted definition. It has been used to describe a deficiency, excess or imbalance of a wide range of nutrients, resulting in a measurable adverse effect on body composition, function and clinical outcome (Elia, 2000).

Malnutrition is defined as 'a state of nutrition in which a deficiency, or excess, of energy, protein and micronutrients causes measurable adverse effects on tissue/ body form (body shape, size and composition) and function, and clinical outcome' (Stratton et al., 2003).

However, in the case of undernutrition, this definition does not take into account the cause of unintentional weight loss. Unintentional weight loss may have three primary syndromes: starvation, sarcopenia and cachexia (Thomas, 2007; Chapman, 2011). Starvation generally occurs as a result of protein energy deficiency and is synonymous with PEM. The main difference between starvation and other syndromes of unintentional weight loss is that it is reversed when adequate energy and protein intake is achieved (Thomas, 2002). In sarcopenia, there is a progressive loss of muscle mass that occurs with normal ageing, though this area is still under investigation (Cruz-Jentoft et al., 2010; Muscaritoli et al., 2010; Fielding et al., 2011). Dietary management alone would be unlikely to address weight loss as sarcopenia is thought to occur regardless of energy balance (Messinger-Rapport et al., 2009; Rolland et al., 2011).

Figure 8.2 shows the status of organ system during severe malnutrition condition. Undernutrition causes the malfunctioning of various body organs, such as the kidney, liver, lungs, intestines, etc. The impaired hepatic function causes loss in

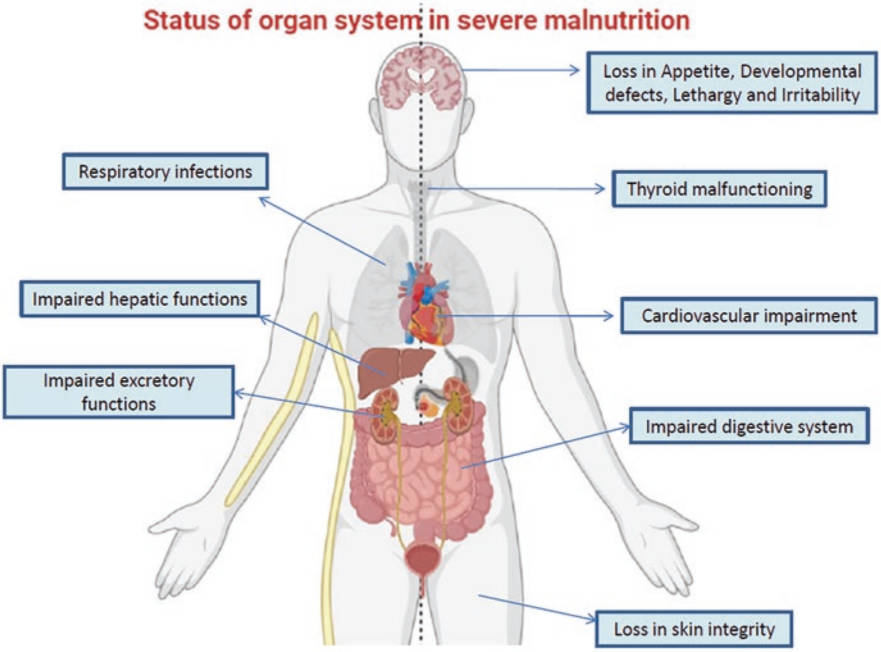

Fig. 8.2 Organ system status in severe malnutrition

appetite and ultimately leads to stunting growth of the individual. Impairment in the cardiac functioning leads to several cardiovascular problems, viz. atherosclerosis, atherosclerosis, etc. Malfunctioning disturbs the digestive system, which directly affects the intestinal functioning of the human body. Other body malfunctioning also takes place in the form of loss in skin integrity and malfunctioning of the neural system.

8.3 Undernutrition

It is the condition where insufficient intake of energy and nutrients causes imbalance in the human body and ultimately leads to a variety of health implications. Majorly, undernutrition is linked with low calorie intake along with deficiency of protein, vitamins and minerals. In most of the cases, undernutrition is interchangeably used with malnutrition. Undernutrition may be found in infants, children, teenagers, adults, and in pregnant and lactating females. Maternal undernutrition is so dangerous that it may cause death of pregnant females and around 800,000 neonatal deaths due to gestational age births, stunting and wasting (Bhutta et al., 2013). Only micronutrient deficiency causes 3.1 million child death annually (Bhutta et al., 2013). Approximately 45% of child death was recorded due to deficiency of vitamin

A, zinc and suboptimal breastfeeding (Black et al., 2013). The deficiency of critical vitamins is found a serious health concern in many diseases, such as autism spectrum disorder (Chong et al., 2022).

Zinc deficiency in children is so critical that it reduces the body's immune response; hence children are at more risk of infectious diseases. In a double-blind, placebo-controlled trials, it was found that zinc was proved to be effective in recovery during infectious diseases when given as adjuvant with antibiotic therapy (Bhatnagar et al., 2012; Walker & Black, 2012). It is also estimated that overweight and obesity increased infant and maternal morbidity (Black et al., 2013). Maternal iron and calcium deficiency is associated with maternal death and low (<2500 g) birth weight (Black et al., 2013). To reduce the problems of undernutrition, infants should feed exclusively initially for 6 months and continue with complementary foods for further 18 months. There has been a greater increase in undernourished people since 2014. Around 630 million people were recorded undernourished in 2014, while 690 million or 8.9% population of the world were recorded undernourished in the year 2019 (FAO, 2019). The coronavirus outbreak in December 2019 exponentially increased the situation till present throughout the world.

The above Fig. 8.3 is showing the starving body of a child clearly depicting high grade of undernourished condition. Somalia is a low socioeconomic country. People living there do not have enough sources of income and access to food. Around the world, African countries are facing huge problems of food security. The coronavirus pandemic has drastically worsened the situation in these regions, although various food security programmes are ran by the World Food Programme and other

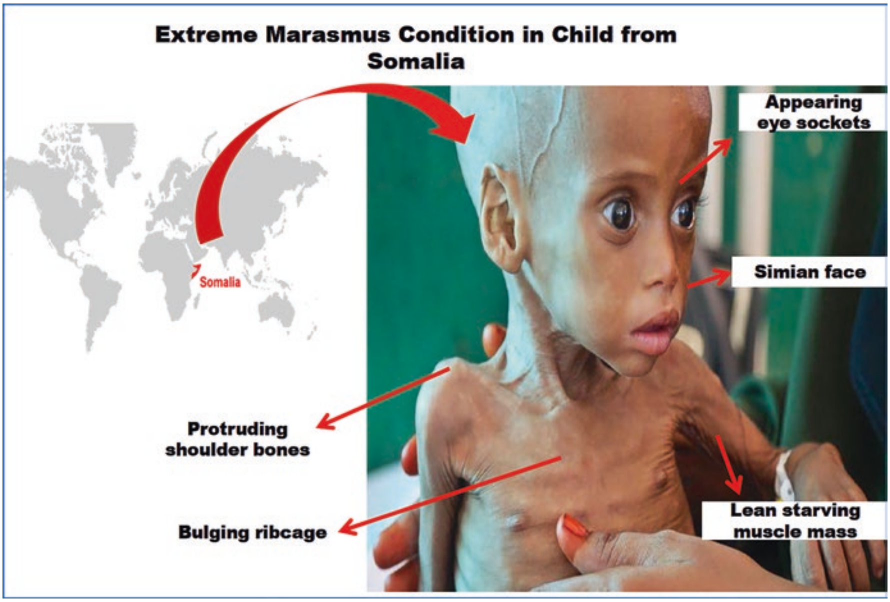

Fig. 8.3 Undernourished child facing extreme marasmus condition. (Source: UNICEF, 2017)

concerned organisation that really help them to alleviate the situation. But still, there is a huge need to work on and to implement a sustainable model to relax the people there permanently or for a longer period of time.

8.4 Types of Undernutrition

There are two main categories of undernutrition as shown in Fig. 8.4, i.e. one is energy undernutrition, which is caused by deficiency of macromolecules in the human body, such as carbohydrates, fats and proteins. Among these, protein energy undernutrition is most significant within the population.

Another class of undernutrition is micronutrient undernutrition, i.e. the deficiency of micronutrients in the human body such as iron, zinc, selenium, iodine, etc. If a person is getting the micronutrients in a diet less than recommended daily allowance, he or she will be suffering from several health implications due to long-term micronutrient deficiency.

The above Fig. 8.5 shows the data recorded during the years 2001–2017. The figure shows clearly that the least developed countries have comparatively greater percent of undernourished population globally and country-wise. It can be seen that the undernourished population was around 30% during 2001. But it slowly declines as it approaches to 2017 due to several factors. In the same years, the least undernourished population was recorded for Latin America and the Caribbean, which starts from 10% in 2001 and slowly declines till 2017. This data was found to be very dramatic in the case of India, as it was recorded as 10% in 2001, while it

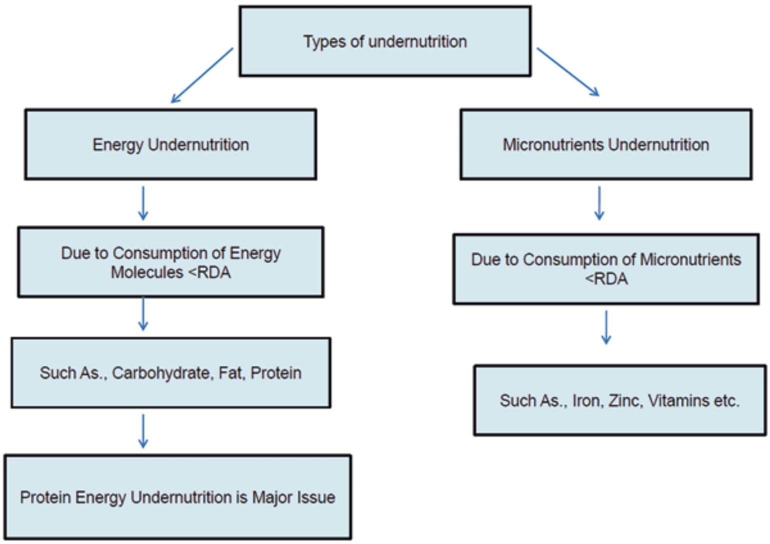

Fig. 8.4 Types of undernutrition

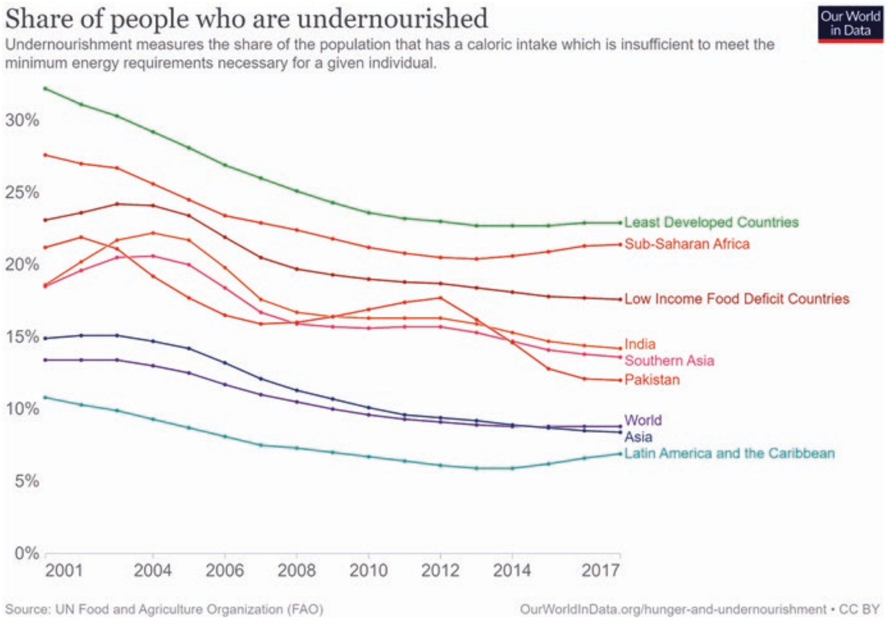

Fig. 8.5 Undernourished populations of world and other countries. (Source: www.ourworldindata.org)

increased till 2004, and from there it decreased till 2017, with little bulge near 2012. The snake pattern was found in the case of Pakistan as it was around 20% in the year 2001. It increased a bit and then went on declining till 2007. From there, it again jumps till 2012 and decreased further till 2017. This type of pattern showed unstable economic conditions within the country.

8.5 Causes of Undernutrition

One of the major contributing factors for undernutrition is the food security problems. However, certain conditions increase the risk of undernutrition. These include being poor, homeless, psychiatric disorders, illness, age-related factors, etc.

There are several causes of undernutrition. The causes are classified under different sections as shown in Fig. 8.6.

Among the different undernutrition types, protein energy undernutrition is the major cause of undernutrition in community. It may happen when a person do not consume enough protein for a longer period of time. Generally, it is observed in countries where food security is a greater issue. Mostly, children suffer from undernutrition problems, and 50% probability of their death is due to severity of

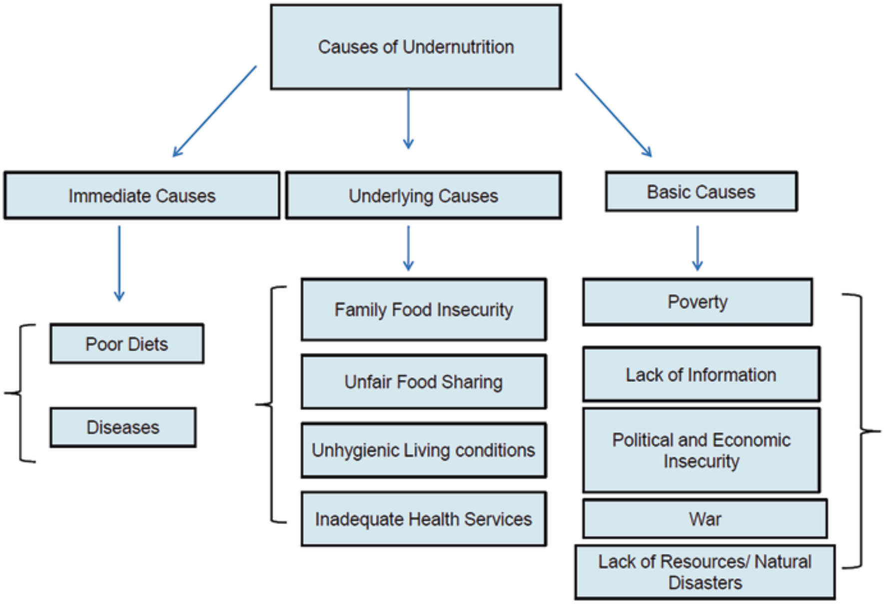

Fig. 8.6 Probable causes of undernutrition

infections. However, irrespective of the age, it can target any individual. Two kinds of protein undernutrition problems are more common, i.e. marasmus and kwashiorkor.

The most dangerous and extreme stage of protein undernutrition is starvation, which results due to partial or total deficiency of essential nutrients for a long period. It usually takes place when there is unavailability of food such as famine or due to anorexia nervosa.

8.6 Consequences of Undernutrition on Human Health

People who are not getting required nutrition may experience weight loss, fatigue, mineral and vitamin deficiency, mood changes, etc. The secondary consequences of deficiency problems may be lethal and may cause life-threatening diseases. Generally, undernutrition problems are found in the children where deficiency of protein, minerals and vitamins take place that put them at high risk of diseases, leading to death in lethal cases. The consequences of undernutrition can be understood in two ways, i.e. short-term adverse effects and long-term adverse effects. The former include recurring illness, delayed physical and mental development, poor appetite, low weight, etc. The deficiency of a particular nutrient causes specific health problems, as discussed in Table 8.1.

Table 8.1 Consequences of undernutrition on human health

S. no.	Deficiency of element		Consequence on human health	References
	Macronutrient	Micronutrient		
1	Protein	–	Kwashiorkor, marasmus, starvation, oedema, hair loss, skin atrophy, impaired multiple organ system, etc.	Morley (2021)
2	Carnitine	–	Impaired muscle metabolism, myopathy, hypoglycaemia, cardiomyopathy	Morley (2021)
3	–	Iron	Anaemia, craving for nonfoods, spoon nails, restless leg syndrome, dysphagia	
4	–	Zinc	Foetal malformations and low birth weight, impaired growth in children, delayed sexual maturation, hypogonadism, alopecia, anorexia, impaired immunity, dermatitis, anaemia, lethargy, impaired wound healing, etc.	
4	–	Vitamin D	Rickets	Johnson (2020)
5	–	Vitamin A	Night blindness, xerophthalmia, dryness of eye and skin, depressed immunity in infants and children, etc.	Johnson (2020)
6	–	Folate	Megaloblastic anaemia, neural tube birth defects in pregnancy	Johnson (2020)
7	–	Vitamin C	Fatigue, depression, connective tissue defects, impaired bone growth in infants and children	Johnson (2020)
8	–	Vitamin E	Haemolytic anaemia, neurologic deficits, skin-related disorders	Johnson (2020)
9	–	Vitamin K	Impaired clotting, haemorrhagic disease of newborns	Johnson (2020)

8.7 Overnutrition

It is the condition that arises due to overconsumption of food. It results in accumulation of fat inside the human body and causes overweight and obesity conditions which ultimately put the human at risk of a variety of health implications such as noncommunicable diseases.

It can be clearly seen in the above Fig. 8.7 that the kwashiorkor child have protruding abdomen with subcutaneous fat along with enlarged and fatty liver. The face of the child is looking round just like moon, with thin and brittle hair, inflammation on body, swollen legs, etc. The ribs are not very prominent with mild growth retardation.

Figure 8.8 shows the types of overnutrition, i.e. energy overnutrition and micronutrient overnutrition. The energy overnutrition is the main concern that is responsible for a variety of noncommunicable diseases as discussed in previous sections. Childhood obesity is the major issue of energy overnutrition in children. It is needed to address at the right time with suitable strategy and plan to check or mitigate the health complications of the children in the coming future. Generally, people feed

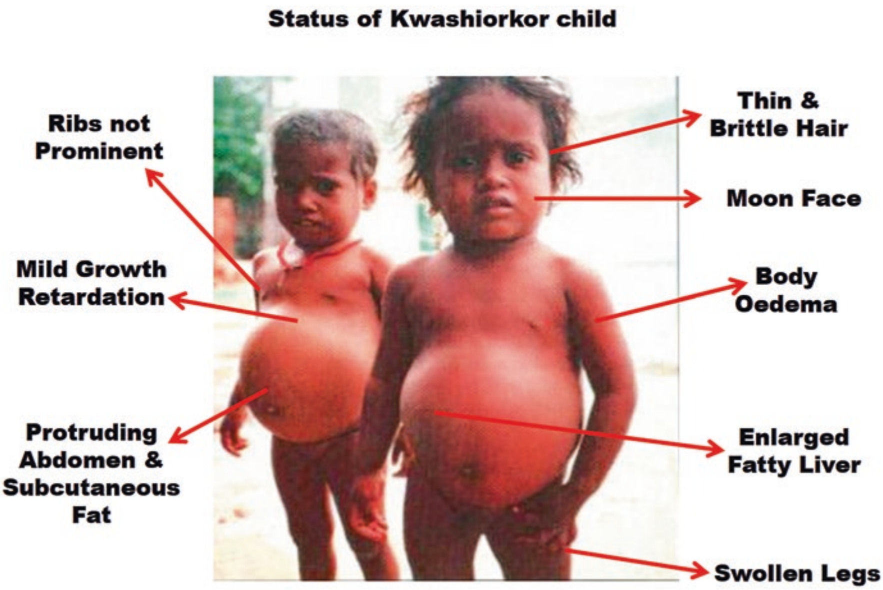

Fig. 8.7 Physical status of kwashiorkor child

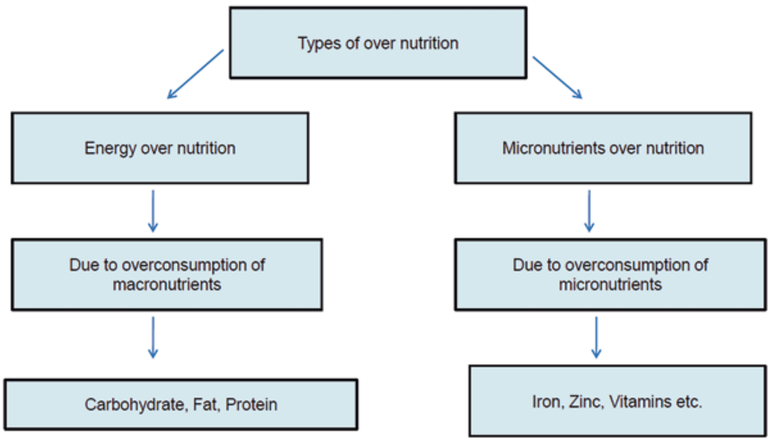

Fig. 8.8 Types of over nutrition

their children with heavy diet due to affection, and this awareless affection causes numerous health problems to their children in the future. So, it is recommended to feed one's children a healthy diet rather than bulky diet. The main alleviating factor of these problems is the awareness, i.e. what to eat and how to eat? Overconsumption of micronutrients such as vitamin A and vitamin D may cause toxicity in the human body as these are fat-soluble vitamins and cannot be elute out through urination and faeces.

8.8 Causes of Overnutrition

The main cause of overnutrition is overeating foods that lead to overweight and obesity in individuals. In many cases, overweight and obesity may be associated with genetics of individual. Overnutrition can be categorized in two broad categories, i.e. energy overnutrition and micronutrient overnutrition. Energy overnutrition means the overconsumption of macronutrients such as carbohydrate, fat and proteins that will result in the accumulation of fat within the body. At the other side, the micronutrient overnutrition is the consumption of micronutrients more than the recommended daily allowance. Finally, it leads to the accumulation of micronutrients such as iron, calcium, zinc, etc. inside the human body and causes the toxicity that may hamper the normal functioning of organs, viz. kidney, liver and lungs functioning, etc.

8.9 Consequences of Overnutrition on Human Health

Overconsumption of food is always more harmful than underconsumption as overconsumption leads to overweight and obesity that ultimately leads to various noncommunicable diseases, viz. cardiovascular disease, hypertension, type 2 diabetes mellitus, colon cancer, polycystic ovary syndrome in females, etc. People, due to different reasons, eat more than they are required and suffer with health complications. Countries which are developed and progressing have more individuals suffering fromnutrition problems such as in the United States, European countries, India, etc.

India stood second in the list of countries affected with type 2 diabetes (from 5.9% in 2000 to 10.4% in 2017) – presently around 72 million people are affected (Swain & Chowdhury, 2018). 1.9 billion adults are overweight or obese, while 462 million are underweight. It is estimated that two third of the global burden of disease is caused by overweight and obesity, i.e. overnutrition. Consequently, hunger is the biggest global health concern, the alleviation of which is cumbersome task. Ultimately, if good management of food consumption will be applies, both overnutrition and undernutrition problems could be minimized. Among the population, childhood obesity is the biggest public health challenge (WHO, 2018). Globally, the problem is not the availability of food resources, but the allocation of food. In 2016, more than 1.9 billion adults aged 18 years and older were overweight. Of these over 650 million adults were obese. In 2016, 39% of adults aged 18 years and over (39% of men and 40% of women) were overweight. Overall, about 13% of the world's adult population (11% of men and 15% of women) were obese in 2016. The worldwide prevalence of obesity nearly tripled between 1975 and 2016. In 2019, an estimated 38.2 million children under the age of 5 years were overweight or obese. Approximately 71% death are recorded due to noncommunicable diseases globally. Among the various noncommunicable diseases, around 17.9 million people die due to cardiovascular diseases. Once considered a high-income country problem, overweight and obesity are now on the rise in low- and middle-income countries, particularly in urban settings. Around 85% deaths of noncommunicable diseases occur in low- and middle-income countries. In Africa, the number of overweight children under 5 has increased by nearly 24% since 2000. Almost half of the children under 5 who were overweight or obese in 2019 lived in Asia.

In all such conditions, people waste a lot of money to rid of these problems, and interestingly, the probability of normalcy is not much high in the case of cancers and cardiovascular diseases. So the sustainable solution is to control the diet by creating awareness among the people along with other preventive measures.

8.10 Therapeutics of Malnutrition

Malnutrition causes millions of death annually throughout the world due to undernutrition complications and overnutrition problems. Although a person suffering with an undernutrition deficiency problems may be treated with adequate food supply that will fulfil the deficiency in the human body, the main concern of treatment with food is the longer period of recovery. That's why, severe patients are treated with medicines or with nutraceuticals, i.e. densely packed doses of nutrients. Currently, lots of pharmaceuticals and nutraceuticals products are available in the market that can treat patients effectively.

The recommended daily allowance (RDA) of various vitamins and minerals at various developmental stages is presented in Table 8.2.

8.11 Sustainable Remedies

It has been discussed the various pharmaceuticals and nutraceuticals remedies to treat malnutrition problems. But this is not a sustainable solution because if the person will not maintain a healthy diet, he or she will have to take these remedies in the future. The ultimate solution for not suffering with malnutrition problems is to add healthier food items in the diet along with an active lifestyle replacing sedentary style.

Currently, people have become much health conscious about their health, and they are opting for healthier food items rather than taking medicines for the same. For instance, it is always better to take vitamin D-rich foods rather than vitamin D tablets.

At the other side, food technology opens fascinating doors for people by making newer food products fortified with different macronutrients, such as proteins, and micronutrients such as iron-rich biscuits, salt, fibre-rich bakery products, vitamin D-rich dairy products, etc.

Table 8.2 Therapeutics of common malnutrition problems for the Indian population

S. no.	Deficiency problem	Remedy	Recommended daily allowance (IU = international unit; μg = microgram)							
			Infants (0–12 months)	Children (4–6 years)	Boys (16–18 years)	Girls (16–18 years)	Men	Women	Pregnant women	Lactating women
1	Vitamin A	Retinol capsules/powder form	350 μg/day	400 μg/day	600 μg/day	600 μg/day	600 μg/day	600 μg/day	800 μg/day	950 μg/day
2	Vitamin D	Cholecalciferol tablets/powder form	400 IU/day	600 IU/day	600 IU/day	600 IU/day	600 IU/day	600 IU/day	600 IU/day	600 IU/day
3	Iron	Dry iron form/iron syrups	3 mg/day[a]	11 mg/day	26 mg/day	32 mg/day	19 mg/day	29 mg/day	27 mg/day	23 mg/day
4	Zinc	Tablets/syrup forms	2.5 mg/day[a]	4.5 mg/day	17.6 mg/day	14.2 mg/day	17 mg/day	13 mg/day	14.5 mg/day	14 mg/day
5	Iodine	Dry form/syrup form	100–130 μg/day	120 μg/day	150 μg/day	150 μg/day	150 μg/day	150 μg/day	250 μg/day	280 μg/day

[a]Taken during 6–12 months; recommended daily allowance as per ICMR-NIN (2020)

So, ultimately, taking fortified food products and replacing refined food items is actually the sustainable remedy to get your health at right track. A variety of fortified food products are available in the national and international market that everybody can go and purchase directly for use.

Globally, research is going on to develop sophisticated fortified food products with higher bioavailability to address the malnutrition issues, and it can be assured that the food scientists and technologists will able to make better food items with affordable cost in coming future.

8.11.1 Awareness Programmes

Although a number of awareness programmes are running by the various national and international organizations, the efficacy is not as it should be. So, there is a thrust to run effective awareness programmes regarding what to eat and when to eat. In India, one such awareness programme is run by FSSAI, i.e. Eat Right India campaign (if it is not safe, it is not food). The governments should start these programmes at the rural level where the prevalence is much higher as compared to the urban places. Several international organisations are also running these programmes such as the WHO, UNICEF, FAO, WFP, etc. Unawareness among the population, is one of the most important factors for undernutrition problems in society, and its prevalence frequency can be mitigated through various awareness programs at national and International levels.

In my opinion, the awareness does not need any platform or organisational structure. But, if the person is willing, that can spread the awareness among the people living in close vicinity. There should be one separate course work of spreading the eat right awareness at school, college and university level, and it should carry some benefits and certificates to the volunteers.

8.12 Government Programmes and Policies

Generally, at domestic as well as across borders, non-governmental organisations are working on these kinds of issues along with some share by government organisations. The awareness of information regarding anything is most important as if the person do not know about the particular fact, how can that be implemented, viz. suppose the latest technology is already developed and available in the market that cannot be utilized by the people without prior advertisement. So, the governments have better infrastructure and command to control things. If it will be led by the governments with sophisticated operating plans, soon, it will reflect good results.

Another aspect is job security; the government should make employment schemes so as to provide their citizen assurance regarding their livelihood.

In India, a lot of schemes are ran by the government under the National Food Security Act 2013, to address the food security issues in the country such as the Midday Meal Scheme, Integrated Child Development Services schemes, Poshan abhiyan, Supplementary Nutrition schemes and Public distribution system.

The government is investing millions of dollars annually on these schemes. But, the needy are getting a fraction of it because of corrupt bureaucratic system. So, to improve the effectiveness, there should be a system so as to bypass the middle men.

8.13 Conclusion

Malnutrition is a major problem in low socioeconomic and developing countries. Malnutrition affects millions of babies, kids, teens and adults, including pregnant and lactating mothers, and it contributes to a high number of deaths worldwide each year. Low per capita income, sometimes known as poverty; a lack of awareness and illness are the main contributors to the problem of malnutrition in society. The burden of malnutrition is increasing mortality and morbidity rates each year. Childhood malnutrition is so widespread right now that it needs to be handled in a more strategic manner to reduce the burden of disease in the future. Researchers must collaborate with medical professionals, social workers and national government partners in order to achieve those goals that are focused on results. The focus must be more towards assuring food security to the people by national and international organisations. In our opinion, the issues must be tackling out locally by domestic government and non-governmental organisations. Even a sophisticated platform is not required in order to work on this. However, one might begin in their immediate area by assisting their neighbours and loved ones. In conclusion, spreading the word about the importance of eating the correct foods for optimum health is crucial. The sustainable remedy is ultimately to improve people's living status by providing them employment and raising their per capita income.

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Chapter 9
Current Perspective on Malnutrition and Human Health

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9.1 Introduction

In India, one of the most important public health problems is malnutrition (World Bank, 2014). Malnutrition is characterised by a lack of essential vitamins and nutrients in a person's diet, as well as a lack, overabundance or imbalance of a variety of nutritional supplements. This has a demonstrably negative impact on body composition, function and clinical outcomes (Elia, 2000). It usually starts in childhood, and if it is not treated on time, it can lead to several health problems, such as physical or mental disorders and weight loss and even may cause death (2019 UNICEF report) (Forms of malnutrition, 2019). In the elderly and people with chronic illnesses, malnutrition is at least two times more common (Stratton et al., 2003). Malnutrition includes several factors that affect human health; some are presented in Fig. 9.1.

MNDs (motor neuron diseases) affect almost two billion people globally (Committee on Micronutrient Deficiencies, et al., 1998). The most prevalent MNDs are iron, iodine, folate, vitamin A and zinc deficiencies. All of these deficiencies frequently contribute to poor growth, intellect destruction and perinatal challenges and increase the risk of morbidity and mortality. The most prevalent form of MND in the world is iron deficiency, which causes anaemia, lowers labour capacity and impairs immunological and endocrine function. Another common condition, goitre, causes iodine deficit as well as psychological or diminished (decreased) cognitive performance. Best immune function depends on adequate zinc intake, and diarrhoea and severe respiratory infections are more common when zinc levels are low. Folic acid is administered early in pregnancy to avoid neural tube abnormalities. Folate is necessary for DNA synthesis and repair, and a lack of it causes macrocytic anaemia.

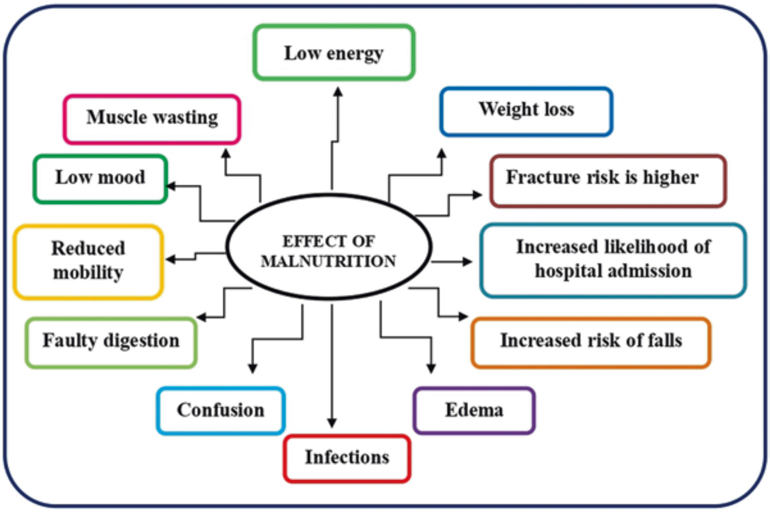


Fig. 9.1 Factors of malnutrition that affect human health

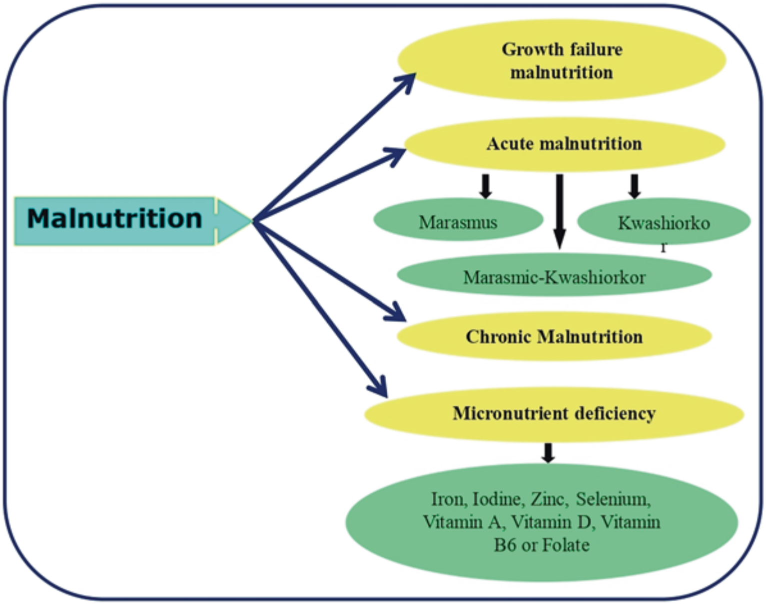


Fig. 9.2 Four different types of malnutrition that cause illness in human health

Worldwide blindness is caused by vitamin A deficiency, which also affects immune system performance and cell differentiation (Bailey et al., 2015). Figure 9.2 lists the four basic forms of malnutrition.

9.2 Forms Associated with Malnutrition

More than 20 million deaths account each year due to undernourishment and micronutrient deficiencies, leading to stunting, wasting, obesity, stunting with obesity and stunting with wasting (Fig. 9.3); it is the leading reason for death and illness in people around the world (Food and Agriculture Organization of the United Nations (FAO), 2017). Additionally, over 800 million people experienced daily hunger (insufficient intake of dietary supplements), and significant micronutrient deficiencies affect approximately two billion individuals worldwide (Food and Agriculture Organization of the United Nations (FAO), 2017a; World Health Organization (WHO), 2018). Despite the fact that over the past 50 years, dietary standards have improved, there has been a decrease in overall hunger among the general population (Food and Agriculture Organization of the United Nations (FAO), 2017).

Hidden hunger is preventable. For the reduction of daily hunger, some new interventions need to be added on the basis of root cause, scope and severity (Bailey et al., 2015). MNDs must be prevented at all costs, and historically this has been done by diversifying diets, fortifying foods and supplementing diets. It is well

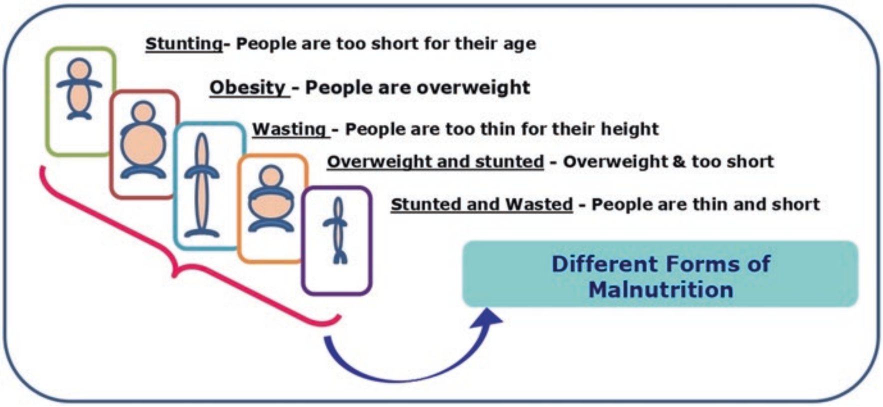


Fig. 9.3 Forms associated with malnutrition

acknowledged that intervention during the first 1000 days is essential for ending the cycle of malnutrition, but it is still necessary to scale up nutrition globally with a coordinated, long-term commitment. It is crucial to comprehend the epidemiology of MNDs in order to identify the optimal interventional techniques for various scenarios (Bailey et al., 2015). Significant deficits in micronutrients affect more than two billion individuals (Food and Agriculture Organization of the United Nations (FAO), 2017; World Health Organization (WHO), 2018). Despite the fact that over the past 50 years, dietary standards have improved, there has been a decrease in overall hunger among the general population (Bailey et al., 2015).

9.3 Deficiency and Essential Nutrient Inadequacies

Worldwide, deficits in iron, zinc, vitamin A, iodine and folate are all far too prevalent (Food and Agriculture Organization of the United Nations (FAO), 2017). In addition, B vitamin deficits, particularly those of B12, are quite frequent (Food and Agriculture Organization of the United Nations (FAO), 2017). Iodine insufficiency affects about 20% of the population in underdeveloped nations, subclinical vitamin A inadequacy affects 25% of children and anaemia affects more than 40% of women (Food and Agriculture Organization of the United Nations (FAO), 2017). Globally, there are around 500 million anaemic women, which raises the mortality rates for both mothers and newborns (Muthayya et al., 2013). Deficits in micronutrients during pregnancy have been related to shortened gestational periods, stunted foetal growth, low birth weight and birth abnormalities in addition to an increased risk of miscarriage, premature delivery and metabolic problems over the long term (Muthayya et al., 2013). More frequently than previously assumed, Menkes disease is brought on by copper deficiency, which predominantly affects the hematologic (anaemia) and neurologic (myeloneuropathy) systems (Altarelli et al., 2019). Over

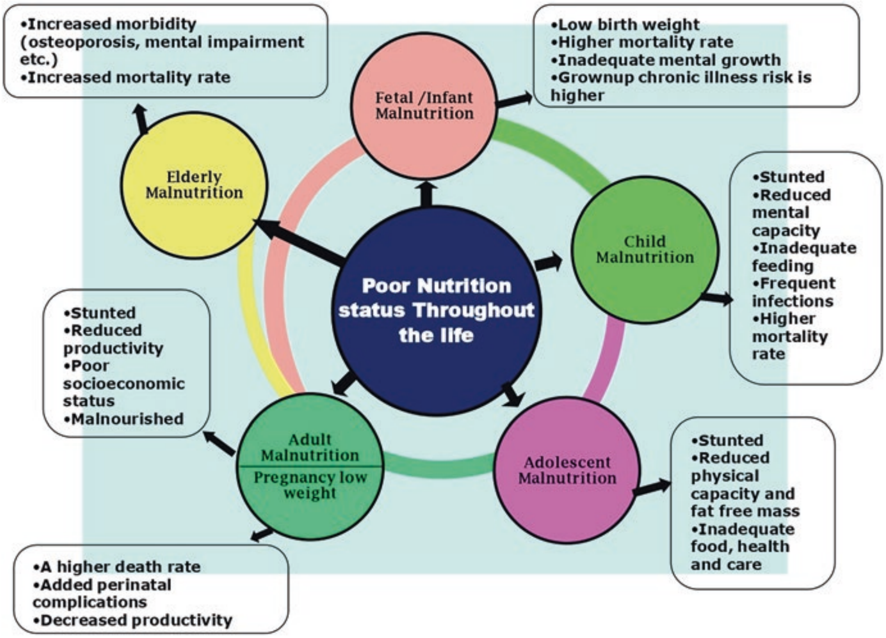


Fig. 9.4 Theoretical foundation for life span cycle of micronutrient deficiencies. (Stoltzfus, 2012)

30% of zinc deficiency affects the majority of people worldwide, and more than 100 countries have recognised vitamin A deficiency as a public health problem (Food and Agriculture Organization of the United Nations (FAO), 2017). According to reports, between 250,000 and 500,000 children worldwide have vitamin A deficiency each year, which is linked to blindness and causes roughly half of those youngsters to pass away within a year of losing their vision (Food and Agriculture Organization of the United Nations (FAO), 2017). Iodine deficiency affects an estimated 38 million newborns and is linked to cretinism, endemic goitre, hypothyroidism, congenital abnormalities and impaired cognitive development and function (Food and Agriculture Organization of the United Nations (FAO), 2017; World Health Organization (WHO), 2018) (Fig. 9.4; Table 9.1).

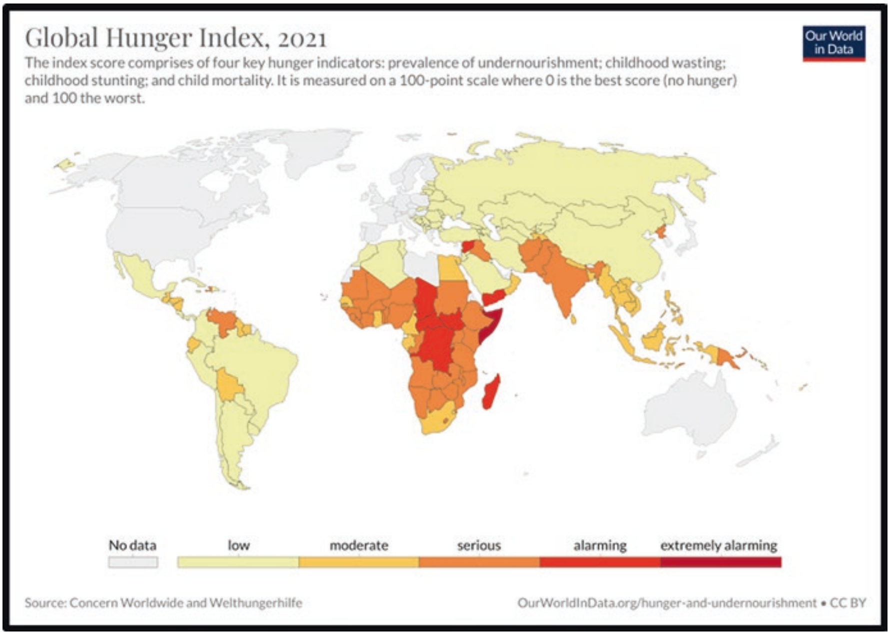
9.4 Global Hunger Index 2021

India scores 27.5 out of 100 in the Global Hunger Index 2021s study by the Welthungerhilfe and Concern Worldwide. The four factors serve as the basis for the Global Hunger Index (GHI), which measures malnutrition, child mortality, wasting and stunting. The GHI is a flawed indicator of "hunger" and therefore does not accurately describe India. Malnutrition is the only one of its four symptoms that are directly related to hunger. In addition to hunger, which is considered a contributing

Table 9.1 Essential micro- and macronutrients

Micronutrients		Macronutrients		
Microminerals	Vitamins	Macrominerals	Amino acids	Fatty acids
Fe	A (retinol)	K	Histidine	Linoleic acid
Zn	D (calciferol)	Ca	Isoleucine	Linolenic acid
Cu	E (α-tocopherol)	Mg	Leucine	
Mn	K (phylloquinone)	S	Lysine	
I	C (ascorbic acid)	P	Methionine	
Se	B_1 (thiamine) B_2 (riboflavin) B_3 (niacin) B_5 (pantothenic acid) B_6 (pyridoxine) B_7 (biotin) B_9 (folic acid) B_{12} (cyanocobalamin)	Na	Phenylalanine	
MO		Cl	Threonine	
CO			Tryptophan	
Ni			Valine	

factor to stunting and wasting in the GHI, stunting and wasting, the other two signs are the results of intricate interactions between various other elements. These other variables include diet, environment, cleanliness and inheritance. Additionally, there is scant evidence to support the fourth indicator, child mortality, as a result of famine.



The GHI report depends on information from international organisations, which are not always up-to-date with what is happening in the nation. The Food and

Agriculture Organization (FAO) of the United Nations, which gathers data on the "Incidence of Malnutrition" indicator, relied on a telephone survey that completely ignored the government's economic response to Covid-19, which includes providing free food to 80 million National Food Security Act beneficiaries through the Pradhan Mantri Garib Kalyan Anna Yojana, and produced an unsatisfactory low estimate for India for the three-year period 2018–2022. The four survey items are unrelated to how much food or energy is in the diet. The FAO estimates of "commonality of undernourishment" in India are 14.8%, 14.5% and 14.0% for the 3-year periods 2015–2017, 2016–2018 and 2017–2019, respectively, showing a clear downwards trend. Afghanistan, Bangladesh, Nepal and Sri Lanka saw improvements in this metric during the 2018–2020 trienniums, indicating that the loss of jobs and money caused by the Covid-19 pandemic had no adverse effects on these countries. India, on the other hand, has not recently faced any significant issues with food availability and production that could have a negative impact on the "prevalence of undernutrition" score. These are incompatible given that Covid-19 will cause more deaths in two countries in 2020. Therefore, the FAO judgement is unreliable and does not deserve respect. The National Family Health Survey (NFHS) is routinely conducted by the government. According to the study, child stunting has decreased from 38.4% (NFHS-4, 2015–2016) to 35.5%, as well as a drop in child wasting from 21.0% (NFHS-4, 2015–2016) to 19.3% (NFHS-5, 2019–2021; NFHS-5, 2019–2021; NFHS-5, 2019–2021). Furthermore, the proportion of overweight kids dropped from 35.8% in 2015–2016 to 32.1% in 2016–2017 (NFHS-5, 2019–2021). Rates of child wasting (17.3%) and child stunting (34.7%) did not change from 2020 and 2021, according to the GHI 2021s study. As a direct focused intervention to address the country's malnutrition issue, the government is putting into action a range of programmes and initiatives with the aid of the Integrated Child Development Services Scheme. All programmes concentrate of these on one or more nutritional factors and have the potential to enhance a nation's nutritional outcomes. The government has made action to avoid malnutrition. With a focus on adopting practices that promote well-being, good health and resilience to disease and malnutrition, the government established the Poshan 2.0 mission to enhance nutrition delivery, outreach and outcomes. In an effort to increase management, there have been advancements in nutritional quality, testing in authorised laboratories, improved supply and technology. The 2006 Food Safety and Standards Act and any corresponding regulations contain criteria for the quality of complementary foods, and the government has issued guidelines to the states and territories to ensure that these standards are met.

To prevent malnutrition and related diseases, it has been recommended that governments and UTs (union territories) promote the use of AYUSH (Ayurveda, Yoga and Naturopathy, Unani, Siddha and Homeopathy) systems. By utilising conventional nutritional knowledge, a programme has been started to close the dietary diversity gap and promote the expansion of Poshan Vatikas in Anganwadi centres. Malnutrition does not directly cause death in children under five. However, by reducing resistance to infections, it can lead to increased morbidity and mortality. The government does not keep a central record of child mortality related to malnutrition because impoverished children are more susceptible to infection than healthy children. Overall under-five mortality decreased from 49.7% to 41.9% between 2015 and 2016.

The National Food Security Act (NFSA) 2013 was passed with the goal of eradicating all hunger. It enables the Targeted Public Distribution System to provide food grains to up to a staggering 50% of urban residents and 75% of rural residents with hefty subsidies (TPDS). There are 81.35 million people living in the country, according to the 2011 census. For families eligible for the Antyodaya Anna Yojna (AAY) programme and priority households, coarse grains, wheat and rice can be purchased for Rs 1/2/3 per kg. AAY homes, in contrast to priority homes, which are only entitled to 5 kg of food per person per month, the lowest of the poor have a monthly entitlement of 35 kg of food per family. All states and territories are now implementing the Act. The scope of the Act is wide to guarantee help to all the weak and needy people of the society (Global Hunger Index, 2021).

9.5 Causes of Malnutrition

Inadequate or excessive intakes of micronutrients along with decreased absorption brought on by various medical conditions such an infection, illness and inflammation are the causes of all forms of micronutrient malnutrition (United Nations Children's Fund (UNICEF), 2013). One form of micronutrient deficiency is under nutrition and an invisible form is referred as hidden hunger (Muthayya et al., 2013). For babies, malnutrition may result from maternal micronutrient deficiency in uterus or rapid postnatal growth (Zlotkin, 2011). Household food instability, poor feeding or care practices and an unfavourable environment with insufficient access to health services are some of the underlying reasons that lead to the immediate causes. Infection, a major contributor to infant mortality (Liu et al., 2012), has a considerable impact on diet (Bhutta et al., 2013a). Infant mortality is most frequently caused by diarrhoea and severe respiratory infections, and MNDs have a significant impact on the immunological response (Bhutta et al., 2013b). Undernutrition is the main contributor of immunodeficiency globally (Katona & Katona-Apte, 2008). Undernutrition has poverty as its fundamental core cause. Even in higher-income countries, some demographic groups still experience micronutrient deficiencies, despite the highest frequency of MNDs in low- and middle-income nations (United Nations Millennium Project, 2000). Macro- and micronutrient requirements rise during pregnancy and lactation (Picciano, 2003). Without receiving the recommended nutritional intake, pregnant women give birth to children with inadequate nutritional status, which can affect their mental and physical development as well as result in stunting, a higher risk of illness and developmental delays (Stoltzfus, 2012). With time, these kids experience nutritional disadvantages as they approach menopause and the cycle repeats itself. Additionally, due to early developmental delays caused by a lack of schooling, persons with nutritional deficiencies frequently have poorer job capacities (ACC/SCN, 2000) (Fig. 9.5).

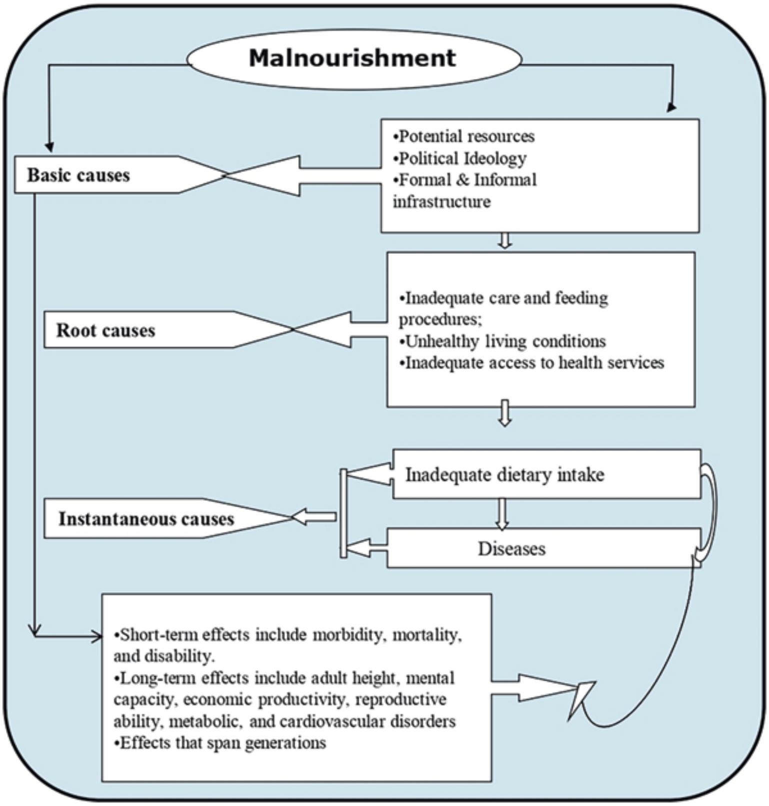


Fig. 9.5 Recent revisions to UNICEF's conceptual framework for the causes of undernutrition. (United Nations Children's Fund (UNICEF), 2013)

9.6 Impact of Malnutrition on Human Health

Micronutrients can have a direct impact on absorption and transport to target cells or organs, or they can have a direct impact on metabolism and organ function via an indirect influence mediated by the microbiota in the digestive system. It is crucial to distinguish between important macronutrients that produce energy and vital micronutrients that do not when discussing human nutrition (trace elements, vitamins, minerals). Micronutrients are crucial for the metabolism of energy, cell division, cell proliferation and immune system health. Numerous researches have looked at how micronutrients affect the host-microbe-metabolic axis and how they affect health. On the other hand, extensive study has been done on macronutrient interactions with the microbiota, notably those impacting the immune system and gut barrier function (Biesalski, 2016) (Fig. 9.6).

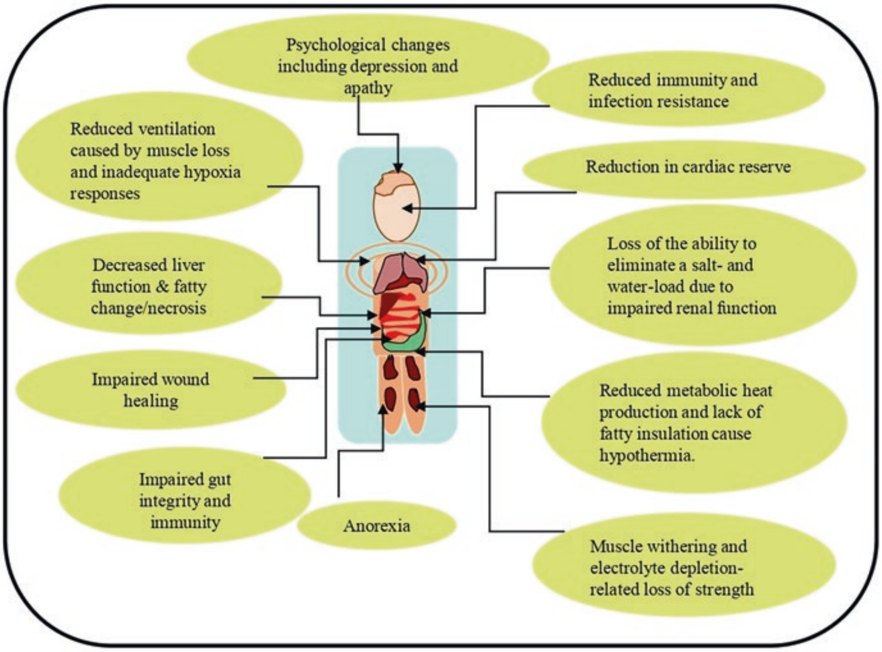


Fig. 9.6 Effects of micronutrient deficiency on human health

9.7 Malnutrition's Consequences

Feeding and nurturing are two of the most basic necessities of all living beings, along with drinking and breathing. For our species to function and survive, we need to eat food that is sufficiently nutrient-dense. However, a sizable section of the global populace is either unable to metabolise and utilise the nutrients available in food or does not have access to enough of it to survive. The malnutrition of these people is present or will develop, which has a number of detrimental effects. In this essay, we will talk about both the concept of malnutrition and its effects. People of any age, race or condition who do not consume enough essential nutrients to maintain the health of their organs may experience serious health effects from nutrient shortage, including death. Although not the only ones, malnutrition can have the following impacts on the majority of persons. On the human body, malnutrition has a number of detrimental effects.

1. *Body weight and volume changes*: Significant weight loss is one of the most immediately obvious signs of malnutrition. However, if starvation persists and affects hormones such as insulin and glucagon, abdominal fat may develop as a result of the altered food metabolism.

2. *Loss of muscle mass*: Without enough proteins, the body tries to defend itself by pulling energy from its own fibres, such as muscle fibres. One of them is protein catabolism.
3. *Hypotonia and a drop in energy*: Lack of nutrients can also cause muscles to become less toned, which lowers their ability to hold tension and exert energy. Additionally, both physical and mental energies are significantly diminished.
4. *Bone fragility*: Malnutrition affects bones similarly to how it affects muscles. They grow more brittle and fragile, increasing the risk of damage and injuries.
5. *Menopause*: Malnutrition also has an impact on the menstrual cycle, causing irregularities and even the cycle's termination.
6. *Immunological system decline*: One of the main organs impacted by nutritional deficiencies is the immune system. Such systems are made vulnerable, leading to a difficult reaction to bacteria and viruses, making infections and diseases much easier to develop.
7. *Dental haemorrhage and irritation*: Gum inflammation and bleeding are symptoms of dental diseases.
8. *Oedemas*: Nutrient deficiencies and electrolyte imbalances brought on by poor nutrition usually cause fluid to accumulate in different parts of the body, causing swellings in the form of oedemas.
9. *Cardiovascular problems*: A lack of nutrients weakens the heart muscle and blood vessels, which can result in arrhythmias, hypotension, heart failure and even death.
10. *A decline in gastrointestinal function*: The digestive system becomes dysfunctional when the body is not receiving enough nourishment to maintain healthy function. In fact, a person who has experienced long-term malnutrition is unable to start eating normally and must gradually adapt in order for his/her digestive motility to return to normal.
11. *A decline in mental ability*: The neurological system is significantly impacted by malnutrition. It is typical to observe changes in cognitive function, such as a decline in the ability to formulate complicated responses, the capacity to plan ahead and make decisions, judgement or the capacity to focus or control behaviour.
12. *Emotional lability, irritability and mental health problems*: A lack of food impairs the ability to control one's behaviour, which facilitates the attack-and-flight response. Emotions can be conveyed more readily than usual. Significantly more frequent are irritability, anxiety and sorrow.
13. *Has an effect on respiratory capacity*: It may have an adverse effect on how well our bodies are able to absorb oxygen and expel carbon dioxide.
14. *It slows down metabolism*: The body makes an effort to lower metabolism in order to conserve energy when it determines that it does not have enough nourishment to function properly.
15. *Hepatic and pancreatic dysfunction*: Malnutrition also has an impact on the liver and pancreas, which are unable to manufacture insulin and glucagon or cleanse the blood, leading to digestive system problems.

16. *Kidney problems*: Damage is also done to the kidneys' capacity to filter blood and eliminate waste and poisonous substances. Lack of nutrients interferes with its ability to filter these substances properly.
17. *Anaemia*: Anaemia, or a decrease in red blood cell synthesis caused by a shortage of fundamental components such as iron or vitamins, is one of the effects of nutrient deficiency. Symptoms include lightheadedness, fainting, migraines, arrhythmias, pallor, numbness and a lack of blood flow in some body parts (Martorell, 2007; Smith & Haddad, 1999; Wisbaum, 2011).

9.8 Childhood Malnutrition

The many repercussions of malnutrition on people, in general, have been covered so far. It has been shown, though, that the evolutionary stage at which malnutrition first appears is crucial. The earlier in the evolutionary process the subject is, the more affectation it will cause. Because of the challenges caused by a lack of nutrients during growth, it will be altered and will not be attained in a regular or predictable manner, requiring you to live with certain consequences for the rest of your life. One of the most serious is child malnutrition, which causes a delay in physical and mental development. For example, when a child's weight and height stop growing, psychomotor delay, speech impairments and attention problems may occur. There are also hair loss and ventral oedema. As brain growth slows, atrophy, a drop in glial cell density and issues with myelination can all happen (Martorell, 2007; Smith & Haddad, 1999; Wisbaum, 2011).

9.9 Microbiota in Malnutrition

A diet that lacks micronutrients but not necessarily energy is characteristic in low-income populations, though this can also be seen in areas of poverty in middle- and high-income countries (Biesalski, 2014). According to predictions, in more than three billion people globally, micronutrient deficiencies are thought to be more prevalent among women and children (mostly Fe, Zn and vitamin A). Two billion individuals are expected to be iron deficient globally; according to the Food and Agriculture Organization/World Health Organization, one billion people may be vitamin A and zinc insufficient, while half a billion may be vitamin D deficient (Keflie et al., 2015). Vitamin D deficiency is a worldwide problem that affects people not only in the northern hemisphere due to insufficient skin's ability to produce vitamin D and insufficient exposure to sunlight but also in places with strong sunlight (Brown & Noelle, 2015). The link between dysbiosis and malnutrition may be explained by vitamin A and D deficiency as well as other micronutrient deficiencies that impair microbial activity. Through interactions with immune cells and microbiota, vitamin A may have a direct or indirect impact on the intestinal immunological response. Induction of transforming growth factor-dependent regulatory T (Treg) cells is enhanced, while pro-inflammatory TH17 differentiation is suppressed (Cha et al., 2010). Vitamin A deficiency has been shown to cause a change in the shape of intestinal cells and the elimination of almost all TH17 cells in the small intestine, greatly reducing the amount of segmented filamentous bacteria (McDaniel et al., 2015). The fact that vitamin A encourages the activation of Treg cells and the homing of imprinted leukocytes to the gut serves as an example of the role that vitamin A plays in mucosal tolerance. Infectious respiratory disorders and gastrointestinal systems are especially associated in children with mild vitamin A deficiency. It has been shown that vitamin A supplements can reduce child mortality by up to 40% (Cassani et al., 2012). When vitamin A-deficient mice were infected with *Citrobacter rodentium*, 40% of mice died, but vitamin A-sufficient mice survived (Biesalski et al., 2001). Recently, numerous authors have fully described additional substantial vitamin A effects on the digestive immune system (Brown & Noelle, 2015; Cassani et al., 2012). Loss of barrier function is a consequence of the (reversible) terminal mucosal epithelial cell differentiation caused by vitamin A deficiency (Biesalski et al., 2001; Biesalski & Nohr, 2004). It appears probable that retinoic acid is accountable for the ability of the intestinal mucosa to act as a barrier and to induce sufficient immune answer. Pathogenic bacteria may be more likely to penetrate the intestinal barrier due to a poor mucosal response caused by vitamin A deficiency, which includes reduced synthesis of mucin and defensin 6 (Sirisinha, 2015). Paneth cells, which are located at the bottom of the crypts and generate defensins, by secreting defensins, and host defence proteins like lysozyme C can regulate the amount of germs on the mucosal surface (Vaishnava et al., 2008). In the intestinal Paneth cells of mice with a modest (subclinical) vitamin insufficiency, we discovered crystalloid inclusions with lysozyme positivity (Koch et al., 1990). Paneth cells can reduce the number of bacteria that are associated with the mucosa, thereby reducing bacterial translocation. To stop germs from spreading from the lumen to intestinal cells, for instance, Paneth cells can produce antibiotic compounds that can be maintained in the mucus layer (Meyer-Hoffert et al., 2008). Under the influence of vitamins A and D, Paneth cells and colonocytes produce cathelicidin (LL-37), a key defensin (Frasca & Lande, 2012). These results emphasise the need of taking enough vitamins A and D to maintain a strong mucosal barrier. The immune system, especially T cell response, lymphocyte activation, proliferation and immune response regulation are all significantly affected by vitamin A, or D deficiency is well known (Mora et al., 2008). The nuclear receptors for vitamins A and D (VDR/RXR or VDR/retinoic acid receptor) heterodimerise to regulate immune system-related gene expression as well as cell growth and proliferation, notably in mucosal epithelial cells. As a result, both vitamins influence host defence through the immune system and intestinal barrier. Studies on vitamin D and intestinal barrier function have found numerous interactions between vitamin A and the intestinal barrier, consistent with the idea that vitamins A and D work together to maintain intestinal barrier function (Cantorna et al., 2014). To maintain appropriate tight junction formation, vitamin D modulates the expression of a number of antimicrobial peptides in DCs, including defensin and cathelicidin (Kong et al., 2008). Iron deficiency anaemia and lactobacillus depletion have recently been linked, and lactobacilli appear to be dependent on vitamin iron (Balamurugan et al., 2010). Iron transporters are found in the right colon and cecum. The growth of bacteria that produce propionate is encouraged by easily fermentable carbohydrates, which boost iron absorption (Levrat et al., 1991). Lactobacilli convert lactate to propionate in fermentation systems, which may be the reason for lactobacilli decline and iron shortage (Ramakrishna, 2013). The effects of the aforementioned micronutrients on barrier function and gut immune regulation may assist to explain the recently discovered impacts of malnutrition on the gut microbiota as well as on health and development.

9.10 Intervention Strategies for Eliminating Malnutrition

Four alternative integrated solutions (strategies) are offered by experts to address the micronutrient shortage: dietary diversity, fortification, biofortification and supplementation (FAO, 2021). Supplementation, a temporary fix for urgent problems, is the direct provision of nutrients in the form of syrup or pills. Fortification, which involves adding micronutrients to basic foods, is a more sustainable strategy. Iodised salt is the most popular kind, but some wheat and milk products are also fortified with different micronutrients (Müller & Krawinkel, 2005). The nutritional value of plant varieties is raised through traditional breeding, genetic engineering (genetic biofortification) and/or fertiliser application in biofortification (agronomic biofortification). Litchi bags enhance the fruit's quality (Lal, 2020). Agricultural goods' nutritional quality is improved by canopy management, the use of biofertilisers and other organic inputs, which eliminates malnutrition and strengthens immune systems. Dietary diversity, or year-round appropriate supplementation of basic staples with fruits, vegetables and animal products, is the most sustainable method of preventing micronutrient deficits. To do this, communities' dietary cultures can be altered through practices including small-scale community gardens, domesticated livestock and canning (FAO, 2021). The aforementioned tactics must be used in tandem with other steps to completely remove micronutrient deficiencies. Health care, education, sanitation, water supply and housing need to be further supported on a governmental level, and education and awareness initiatives need to be extended at the same time. Newly useful information can be discovered by in-depth study of science and technology (Joachimiak, 2021). Future obstacles will need to be overcome, such as those brought on by population expansion and climate change (Gibson, 2011). A multifaceted strategy involving society, politics and science is thus necessary for the sustainable control of micronutrient deficiencies (Caulfield et al., 2006) (Fig. 9.7).

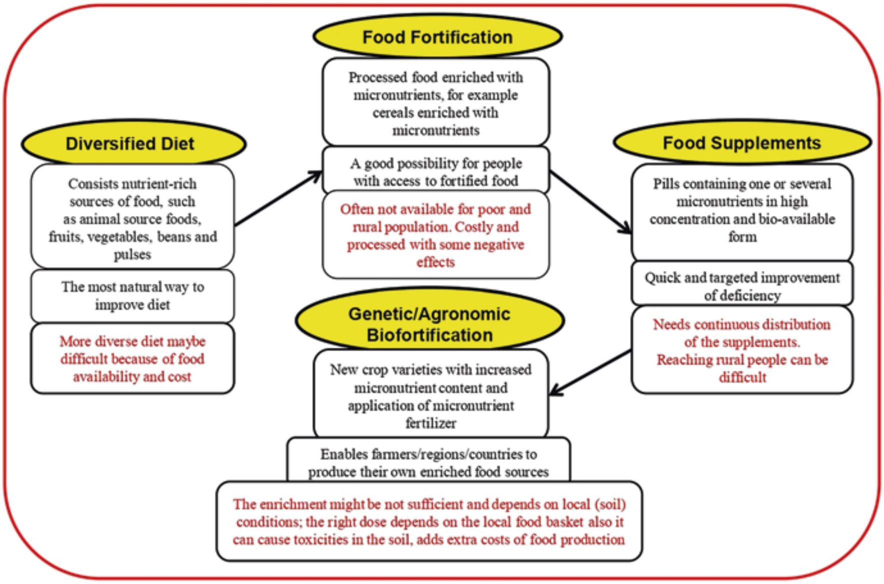


Fig. 9.7 The main methods for addressing micronutrient deficiencies, along with examples, benefits and drawbacks of each method

9.11 Eliminating Malnutrition Through Horticulture

Malnutrition is a global issue that affects both developed and poor nations. It is mostly caused by excessive population growth, low food grain productivity, low incomes and also a number of other social, cultural and economic reasons. It significantly increases the risk of illness, disease infection and reduced immunity. Malnutrition is a riskier problem in developing countries in Asia and Africa. Malnutrition can take many different forms, such as protein energy malnutrition (PEM), which is characterised by the appearance of the skin and bones, and covert hunger. Malnourished individuals may appear healthy on the outside but be weak on the inside. Due to the absence of vital vitamins and minerals from the daily menus, hidden hunger appears as a disorder even when the body is consumed by it. Only the use of micronutrients can suppress a person's hidden hunger. According to the Indian Council for Medical Research (ICMR), we need 280 g of vegetables and 120 g of fruits per day (100 g of leafy vegetables, 100 g of fruits and 80 g of root vegetables). Fruits and vegetables are referred to as protective foods because they are rich in essential micronutrients (vitamins, natural antioxidants and minerals) that improve immunity and metabolic processes, strengthen a person physically and mentally, maintain fitness and prolong a person's life organs. Several vegetables and fruits are also high in carbohydrates. The body converts carbohydrates into the simplest form of sugar, which provides us with the energy and calories we need for a healthy body to function. Excellent sources of carbohydrates include vegetables such as potatoes, sweet potatoes, sugar beets, radishes, peas and fruits such as banana, avocado, dates, sapota, mango, guava and jackfruit (Tables 9.2 and 9.3). Fruits and vegetables including peas, cowpeas, kidney beans, amaranths and others are excellent sources of protein, as are nuts such as cashews, almonds, walnuts and pistachios (Tables 9.2 and 9.3). A healthy adult diet should contain more than 80 g of fat each day. Fruits and vegetables are usually low in fat, although some fruits, such as walnuts (64.50%), almonds (58.90%), cashews (46.90%) and avocados (22.80%). Fruits and vegetables are great sources of fibre and make them easy to eat. Many fruits and vegetables, including amaranths, mustard greens, beet greens, spinach and pomegranates, as well as aonla, grapes, walnuts and others (Table 9.2), are high in fibre. To improve digestion and prevent problems with constipation, an adult should include at least 40 g of fibre in his/her daily eating habits. The main sources of provitamin A, vitamin B, vitamin C, vitamin E and folic acid are fruits and vegetables. In the human body, provitamin A can be easily converted to vitamin A. Fruits and vegetables provide most of the vitamins A and C that people need in their diet. The average human excretes 20–30 g of salt-containing minerals per day, including chlorides, phosphates, sulphates, potassium, sodium, calcium and magnesium. Therefore, it is necessary to maintain these losses with a regular intake of the right amount of mineral salts. For the healthy growth and development of newborns and children, various vital minerals are especially needed (Lal, 2020).

Table 9.2 Values of essential fruits in terms of nutrition and medicine (per 100 g edible portion)

Fruits	Carbohydrate (g)	Protein (g)	Fibre (g)	Vitamin A (IU)	Vitamin B_1 (mg)	Vitamin C (Mg)	Calcium (Mg)	Iron (mg)
Mango	17.00	0.51	1.80	3894	0.058	27.7	10	0.40
Banana	23.43	1.03	2.40	81	0.045	12.0	6	0.6
Pineapple	12.39	0.39	1.20	23	0.092	15.4	7	0.37
Papaya	9.81	0.61	1.80	1750	0.027	61.8	24	0.10
Guava	11.88	1.00	5.40	792	0.050	183.5	20	0.31
Sapota	19.96	0.44	5.30	60	0.000	14.7	21	0.80
Jackfruit	24.01	1.47	1.60	297	0.030	6.7	34	0.60
Litchi	16.53	0.83	1.30	–	0.011	71.5	5	0.31
Grapes	17.77	0.66	1.00	73	0.092	10.8	11	0.26
Avocado	7.39	1.98	5.00	612	0.108	7.9	11	1.02
Aonla	13.70	0.50	3.40	91	0.030	600	50	1.20
Ber	17.00	0.80	–	70	0.020	76	4	0.50
Pomegranate	17.17	0.95	0.60	–	0.030	6.1	3	0.30
Custard apple	23.50	1.60	3.10	–	0.070	37	17	1.50
Fig	19.18	0.75	3.30	142	0.060	2	35	0.37
Phalsa	14.70	1.30	1.20	698	–	22	129	3.10
Jamun	15.56	0.72	0.90	3	0.006	14.3	19	0.19
Lime	10.54	0.70	2.8	10	0.030	63	33	0.60
Lemon	9.32	1.10	2.8	29	0.040	53	26	0.60
Orange	11.75	0.94	2.4	205	0.087	53.2	40	0.10

Table 9.3 Important vegetable's nutritional and therapeutic benefits (per 100 g edible portion)

Vegetables	Protein (g)	Carbohydrate (g)	Calcium (mg)	Iron (mg)	Carotene (mg)	Thiamine (mg)	Vitamin C (mg)
Amaranths	4.00	6.10	397.00	25.50	5520.00	0.03	99.00
Spinach	3.40	6.50	380.00	16.20	5862.00	0.26	70.00
Fenugreek	4.40	6.00	395.00	16.50	5862.00	0.26	70.00
Cabbage	1.80	4.60	39.00	1.80	1200.00	0.06	124.00
Drumstick leaf	1.80	4.60	395.00	16.50	2340.00	0.04	52.00
Mint	4.80	6.00	200.00	15.60	1620.00	0.05	27.00
Turnip	1.70	5.80	18.00	1.00	–	0.04	10.00
Onion	1.20	8.80	47.00	0.70	–	0.08	11.00
Radish	0.70	11.10	35.00	0.40	3.00	0.06	15.00
Sweet potato	1.20	3.40	46.00	0.80	6.00	0.08	11.00
Bitter gourd	1.60	28.20	20.00	1.60	126.00	0.07	88.00
Tomato	0.90	3.60	48.00	0.40	351.00	0.12	27.00
Brinjal	1.40	4.20	18.00	0.90	74.00	0.04	12.00
Cauliflower	2.60	4.00	30.00	1.50	30.00	0.04	56.00
Caw pea	3.50	8.10	72.00	2.50	564.00	0.07	14.00
Drumstick	2.50	3.70	30.00	5.30	110.00	0.05	120.00
French bean	1.70	4.50	50.00	1.70	132.00	0.08	24.00
Okra	1.90	6.40	66.00	1.50	52.00	0.07	13.00
Pea	7.20	15.90	20.00	1.50	83.00	0.25	9.00
Pumpkin	1.40	4.60	10.00	0.70	50.00	0.05	2.00
Ridge gourd	0.50	3.40	18.00	0.50	33.00	–	5.00
Snake gourd	0.50	3.30	26.00	0.30	96.00	0.04	–
Potato	–	–	10.00	0.40	24.00	–	17.00

Despite the production of fruits and vegetables in India being the second highest in the world, malnutrition is still a serious issue there since some people are extremely poor and unable to buy nutritious fruits and vegetables, even though these individuals rely solely on unnutritious dietary grains. These groups of people can be fed nutritionally fruits by growing fruit crops on the road side. Generally, both sides of the road are used for plantation of forest tree, and fruit crops, especially stress tolerance and underutilised fruit crops such guava, jamun, ber, bael, chironji, sapota, phalsa, aonla, kher, custard apple, etc., can be easily grown and made available to the poor who cannot buy from markets (Lal, 2020).

9.12 Malnutrition Reduction Methods Currently Used in Developing Nations

Deficits in diet affect a large portion of the population in the developing world, which has an impact on both people's health and the economies of the nations in which they live. Globally, nutrient-balancing techniques include food fortification and dietary supplementation. Despite this, given the sometimes poor accessibility of rural communities and people living in developing nations, they are challenging to address successfully over the long run. Food fortification, a method of enhancing and supplementing processed foods, entails a minor adjustment to the system of food distribution that is already in place. The same portion sizes are maintained, but by adding important macro- or micronutrients that are necessary for both physical and mental health functions, the portions are made more nutrient-dense. It has been demonstrated that using food fortification techniques in nursing homes is advantageous and economical (Peumans & Van Damme, 1995). Fortification techniques are particularly effective when applied to goods that are previously consumed in nursing homes. To successfully reach the recommended vitamin D levels, fortified milk consumption has been employed for 6 months (Cave et al., 2019; Grieger & Nowson, 2009). As a result, there were improvements in muscle strength, calcium digestion and bone quality (with supplementary vitamin supplementation). Food fortification, therefore, provides a long-term solution to the issue of natural ageing and the resulting undernutrition. This demonstrates how dietary fortification can enhance the quality and variety of meals, particularly when combined with nutrient-dense fruits and vegetables. The beneficiaries are not always as simple to reach, and canning food results in significant vitamin loss. In addition, industrial food processing frequently adds sugar, fat and sodium, which can lead to obesity, hypertension and/or diabetes. Due to the rising problem of obesity brought on by the use of industrially processed meals, it does take a lot of time and money to adopt fortified food, especially in areas with weak infrastructure (Grieger & Nowson, 2009; Pinstrup-Andersen, 2007) (Fig. 9.8).

9.13 Tackling the Micronutrient Malnutrition

In some circumstances, dietary supplements, food fortification and education have all shown promise in reducing malnutrition; these approaches will remain essential. Iodised salt-based dietary supplementation programmes have been successful in a number of countries, for instance. The low-cost initiatives target many of the most at-risk groups (Hetzel, 1990). Similar initiatives for the micronutrients Fe, Zn and vitamin A, however, are pricey, need yearly investments and are unlikely to reach all at-risk populations. Moreover, for logistical, political and economic considerations, these intervention projects have frequently been halted (Gibson, 1994). Part of the remedy to micronutrient shortages, according to nutritionists, is persuading the

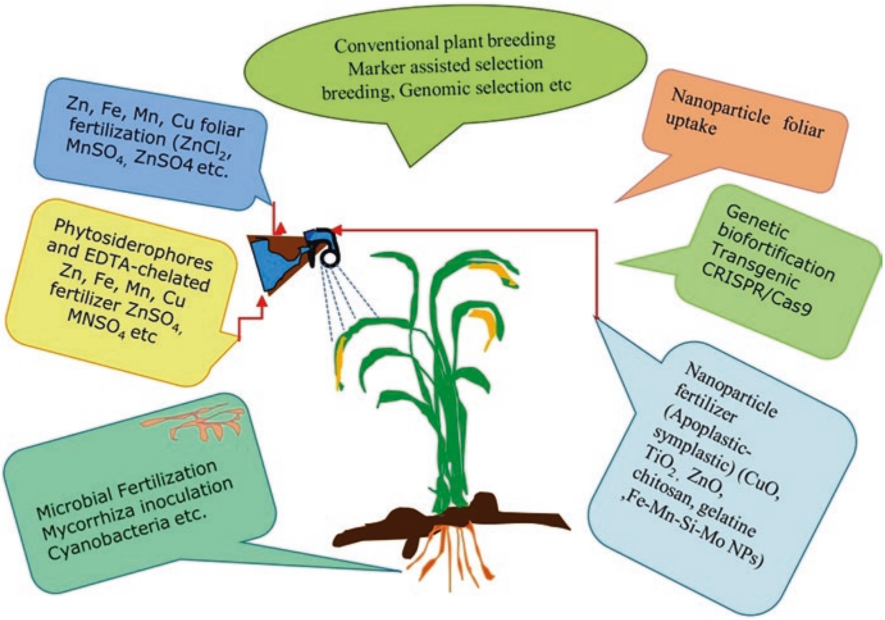


Fig. 9.8 Different approaches of biofortification

public to eat more nutritious foods. Attempts to improve eating habits have been fruitless thus far. If you are poor, it can be tough to make nutritional modifications utilising local items. In Northeast Thailand, a project aimed at increasing vitamin A consumption among the impoverished yielded promising effects. The study focused on the ivy gourd (*Coccinia grandis*), a locally grown vegetable that is high in vitamin A and simple to grow, in order to highlight the significance of vitamin-rich meals to devoted and caring mothers. Most diet-change programmes, on the other hand, result in participants returning to their former habits. Such strategies have only been successful in a few instances. They necessitate a lot of input, as well as continual monitoring and teaching. They rarely work when scaled up; therefore they are unlikely to be sustainable. Breeding for seeds rich in trace minerals has been found to be the most successful method of addressing micronutrient deficiencies under these restrictions. Crop varieties with seeds rich in minerals are not only useful for reducing covert hunger but also for growing on soils deficient in trace minerals. Research from Australia and elsewhere has shown that seeds with higher concentrations of a particular micronutrient had increased germination, seedling vigour and infection resistance during the delicate seedling period. Better grain yields may result from these crop establishment benefits. The priorities of human and plant nutrition may therefore frequently coincide (Graham & Welch, 1996). The new method for supplying micronutrients to the underprivileged in developing countries involves utilising both biotechnology and conventional plant breeding to improve the nutritional value of the staple foods they consume. This technique is low-cost and long-term, as it does not necessitate a change in dietary habits or the ongoing costs associated with fortification and supplements. Breeding micronutrient dense staple crops (biofortification) that feed the world's poor has the greatest promise for improving nutritional status on a global scale. Several crops are undergoing biofortification efforts.

9.14 Conclusion

A robust approach to food, agriculture and health can offer a long-term remedy for unmet hunger. Subtle hunger can be managed with strategies including dietary variety, supplements and strengthening. Combining conventional breeding with genetic-assisted selection and genetic engineering technologies will make it simpler to breed desirable cultivars with better yields, concentrated micronutrients and improved bioavailability of micronutrients in breeding biofortification systems. For biofortification initiatives to be truly successful, nutrition education, processing procedures and home visits are necessary. To address the threat of covert hunger, consistent governmental support for scientific endeavours is important. The food will be safe for the consumer's health and for the identification of biofortified plants thanks to regulated authorisation. The bioavailability of the nutrients provided by biofortified plants requires additional research efforts. There is no one-day effort, personal plan or method that can completely eliminate hidden hunger. Biofortification provides possible ways to reach malnourished people in remote rural areas, bringing naturally nourished food to people. A comprehensive strategy is needed that includes a variety of adaptive interventions in certain countries and regions.

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Chapter 10
Indian Saffron Use as a Source of Drugs and Therapeutics

Rupali Behera, Siddhartha Shankar Sharma, Jannila Praveena, Bamerishisha Rymbai, and Laxmipreeya Behera

Contents

10.1 Introduction

In the culinary setting, turmeric is used as a flavouring agent to improve food odour, medicinal properties, and economic importance. It contains high levels of polyphenols, polysaccharides, and alkaloids (Chempakam & Parthasarathy, 2008) and with other antimicrobial agents used for the development of antimicrobial skin gels and emulsions with improved skin protection and wound dressing properties. The

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largest markets for turmeric are to be found in Japan, Sri Lanka, Iran, the UAE, the United States, the United Kingdom and Ethiopia (). In India, the principal mainly turmeric-growing or -producing states are Telangana, Andhra Pradesh, Tamil Nadu, Karnataka, Odisha, West Bengal and Maharashtratra. The 2018–2019 statistics show that in the amount of land devoted to crop production is increasing. For example, in Telengana 47,888 ha were covered by the government, compared to 44,956 ha last year.

Nowadays knowledge of ayurvedic values are decreasing due to poor awareness among peoples. And the majority of the people are consuming chemical, synthetic pharma based medicines, which have a lot of side effects. Curcumin (diferuloylmethane, 1,7-bis[4- hydroxy-3-methoxyphenyl]-1,6-heptadiene-3,5-dione is one of the components of *Curcuma longa* of yellow colour, which is extracted by its root and has a variety of therapeutic effects. The rhizome is an underground stem that is thick and fleshy, and ringed with the bases of old leaves; these are all parts of turmeric which possess potential medicinal properties. Curcumin is a nontoxic, highly promising natural antioxidant compound which has a wide range of biological functions. Curcumin is now also available in pure form, which shows a wide spectrum of biological activities; it would be easier to develop new drugs from this compound after extensive studies on its mechanism of action and pharmacological effects. It is anticipated that curcumin may find some application as a novel drug in the near future to control various diseases and disorders as well as oxidative stress.

Curcumin may also have potential as an anticancer agent. Its effects appear to be diverse and they are likely to modulation have an impact in terms of cell survival. Studies have shown that curcumin has been known to interfere with various biochemical pathways involved in cancer cell proliferation and survival.

10.1.1 Turmeric

The *Curcuma longa* variety of "Turmeric" or "Indian saffron", which is an herbaceous medicinal plant, belongs to the Zingiberaceae family. Turmeric's native place is tropical Southeast Asia. As stated above, it contributes not only as a colouring agent in food but also has medicinal properties. It consists of large leaves with yellow flowers. The yellow colour Curcuma is extracted by its root and gives therapeutic effects. It is also used in a traditional way as curcumin powder on skin, lactating mothers use it by mixing in milk, it is also used as a spice in cooking and it is mixed with aloe-vera gel to act as an antimicrobial agent in apparent infections. It carry antidiabetic, antibacterial, antifungal, antiprotozoal, antiviral, antifibrotic, antivenom, antiulcer, hepatotoxicity, hypotensive and hypocholesteremic etc. like nature. It reduces high plasma cholesterol and also helps to protect from antiplatelet activity to heart and vessels and also to protect against DNA damage in lymphocytes.

Turmeric has a range of different types of varieties of different places in India, as detailed in Table 10.1.

Table 10.1 Different varieties of turmeric

Sl. No.	Varieties of turmerics	State	Reference
1	Allepey Finger	Kerala	B. Jyotirmayee and Gyanranjan Mahalik
2	Rajapore, Sangli	Maharastra	”
3	Nizamabad Bulb	Andra Pradesh, Telangana	”
4	Duggirala	Andra Pradesh	”
5	Erode local, Roma, PTS-10, Suguna, Sudarsana, BSR-1, Salem	Tamil Nadu	”
6	Lakadong	Meghalaya	”
7	Lokhandi	Maharastra	”
8	Kasturi, Armoor, Chaya	Andra Pradesh	”
9	Dughi, Jobedi, Katigia, Roma, Ranga, Rasmi, Suroma	Odisha	”

10.1.2 The Lakadong Variety of Turmeric

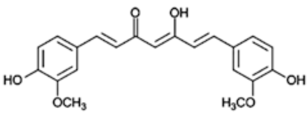
Curcuma longa var. Lakadong is originated from the Lakadong area of the Jaintia Hills district of Meghalaya. This region produced 10,509MT of turmeric from 1817Ha in 2005–2006 (Daimei et al., 2012). Lakadong turmeric contains approximately 6.8%–7.5% curcumin, making it the strongest source in the world. Sometimes, this turmeric variety qualifies in the Geographic Indicator list of India. Its rhizomes are slightly dark in colour and it has many medicinal properties. It is used in the production of drugs, dye, fungal infection re medication chloramphenicol and restore to health or rehabilitate for chronic diseases etc.

10.2 Biological Properties

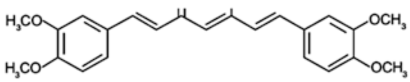
1. Antioxidant

Turmerin is a heat-stable, noncyclic peptide of 40 amino acid residues that was identified as a water-soluble peptide (5-kDa). Srinivas et al. (1992) found it to be an effective antioxidant, DNA protector, and antimutagen. Turmerin has three methionine residues, which contribute to its antioxidant properties. Curcumin has a phenolic structure and a diketone derivative that protects against the damaging effects of oxidative stress. Curcumin has antioxidant characteristics similar to Vitamin C and Vitamin E, reducing lipid peroxidation by protecting antioxidant enzymes, including SOD, CAT, and GPx.

Turmeric extract prevented atherosclerosis and led to a decrease in LDL cholesterol. Meta-analysis research has shown that curcuminoids have been demonstrated to have a significant influence on serum SOD and CAT activity, GSH concentrations, and serum lipid peroxides in a. Curcumin supplementation enhances systemic antioxidant capacity, lipid peroxidation, and inflammation biomarkers in people with metabolic syndrome, according to a study that backs up this claim (MetS). Curcumin has been demonstrated to improve systemic oxidative stress indicators by modulating GSH activity. Inn a previous study, supplementing curcuminoids with piperine lowered the rate of proxidant/antioxidant levels in obese people at high risk of MetS. Another study found that an eight-week supplementation with a lecithinised curcuminoid preparation (180 mg/day) improved serum SOD activities as well as other antioxidant indices, such as serum CAT activities and reduced glutathione and thiobarbituric acid reactive species concentrations in patients with tumours. Curcumin is also a lipophilic molecule, making it an effective scavenger of peroxyl radicals, similar to vitamin E.



Curcumin



Dimethoxycurcumin

2. Anti-inflammatory

Arachidonic acid metabolism was suppressed and anti-inflammatory action was reported as a result of the inhibition of cycloxygenase and lipoxygenase enzymes in the intestinal mucosa by the addition of curcumin to the diet. Turmeric has been found in studies to minimise the detrimental effects of inflammatory substances like leukotriene, prostaglandin, tumour necrosis factor, and interleukin. The combined anti-inflammatory action of powdered turmeric and linden was found to be as efficient as cortisone in carrageenan-induced edoema in one investigation. Curcumin decreases inflammatory markers, improves glucose metabolism, and reduces weight and waist circumference, according to (Chuengsmarn et al., n.d.) (Fig. 10.1).

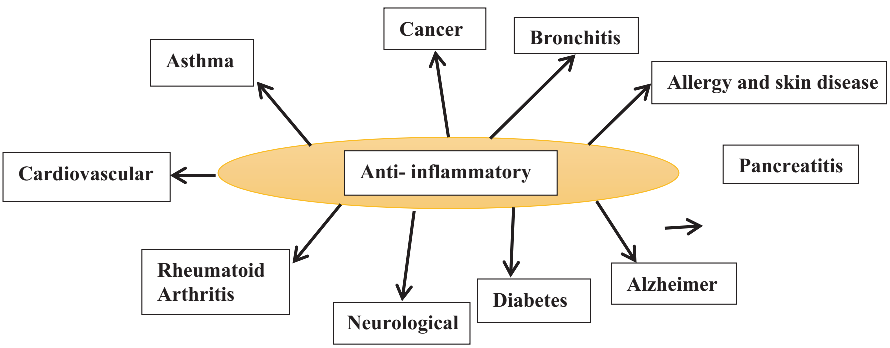


Fig. 10.1 Curcumin as an anti-inflammatory in different diseases

3. Anti-Bacterial

Kumar et al. (2001) discovered that an aqueous extract of turmeric rhizomes had antimicrobial properties. In vitro, curcumin inhibits the development of Helicobacter pylori CagA+ strains. Curcumin and the oil fraction both inhibit the growth of germs such as Streptococcus, Staphylococcus, Lactobacillus, and others.

Curcumin has also been shown to suppress the growth of periodontal bacteria and Porphyromonas gingivitis Arg- and Lys-specific proteinase (RGP and KGP, respectively) activities in antibacterial studies. Curcumin also inhibited the production of Streptococcus gordonii biofilms and P. gingivitis homotypic biofilms in a dose-dependent manner. At relatively low quantities of curcumin, bacterial growth was almost entirely inhibited. P. gingivitis biofilm development was suppressed by more than 80% at a dosage of 20 g/mL curcumin. Curcumin at 100 g/mL, on the other hand, did not inhibit Aggregatibacter actinomycetemcomitans from growing. Curcumin also targets bacterial membranes (Escherichia coli) when used at relatively high concentrations (Alsamydai & Jaber, 2018).

4. Anti-fungal

Substances and extracts obtained from various natural resources, particularly plants, have long been a valuable tool for combating fungal infections and deterioration. Curcumin has been studied for its ability to reduce fungal spoilage and fungal infections as a result of its widespread traditional use in food products. Ether and chloroform extracts and oil of turmeric have antifungal effects (Apisariyakul et al., 1995). Crude ethanol extract also possesses antifungal activity. Turmeric oil is also active against Aspergillus flavus, A. parasiticus, Fusarium moniliforme and Penicillium digitatum (Jayaprakasha et al., 2006).

5. Anti-Viral

The lack of effective medicines for most viral diseases, as well as the growth of antiviral medication resistance and the high cost of existing antiviral therapies, necessitates the development of novel antiviral agents. Furthermore, current antiviral medications are not always well tolerated, efficacious, or satisfying. As a result, the growing demand for antiviral medicines will be highlighted still further. Scientists are interested in plants because they contain large amounts of phytochemicals with various biochemical pathways, especially antiviral activity.

Curcumin is antiviral (Araujo & Leon, 2001). It is an effective Epstein-Barr virus inhibitor (EBV). Curcumin is also anti-HIV (human immunodeficiency virus) because it inhibits the HIV-1 integrase, which is required for viral replication. UV light is also blocked. It enhanced the expression of HIV genes. As a result, curcumin and its analogues could be used to produce innovative HIV drugs.

6. Anti-Cancer

Cancer is the second-highest cause of mortality in the world, claiming the lives of more than six million people each year. Many important medications have been discovered as a result of scientific examinations of plants utilised in various types of ethnic medicine, including taxol, camptothecin, vincristine, and vinblastine. Curcumin has been shown to have anticancer properties, whether used alone or in conjunction with traditional chemotherapy medications, to treat cancer and cancer-related problems in numerous trials. Curcumin inhibits carcinogenesis by altering two key processes: angiogenesis and tumour growth, according to in vitro and in vivo studies. Curcumin has shown to be an effective anticancer and antifungal agent when used alone or in combination with other anticancer and antifungal medications.

Curcumin suppressed the development of human cancer cells by inducing apoptosis in a dose- and time-dependent manner, which was accompanied by apoptosis induction. Curcumin's anti-tumour action is mediated by its antiproliferative effect in a variety of cancers, inhibitory action on transcription factors and downstream gene products, modulatory effect on growth factor receptors and cell adhesion molecules involved in angiogenesis, tumour growth, and metastasis, and recent research has suggested that curcumin's antitumor potential could be mediated by telomerase inhibition.

7. Anti-diabetic

Curcumin has anti-diabetic properties, according to research. Curcumin's antioxidant properties may be responsible for its anti-diabetic properties. Researchers found that curcumin had a favourable effect on diabetes-induced endothelial dysfunction by lowering superoxide generation and inhibiting vascular protein kinase C in their investigation. Curcumin has recently been shown to have the ability to directly quench reactive oxygen species (ROS) that can lead to oxidative damage in recent studies.

8. Antivenom

Antivenom is a composition of antibodies which protect from significant toxicity or a high risk of toxicity. Curcumin is also having such ability according to studies. The structural link between medicinally essential herbal substances such as acalyphin, chlorogenic acid, stigmasterol, curcumin, and tectoridin, as well as PLA2 from Russell's viper, was investigated. The molecular modelling investigations demonstrated that the peptides at the active site of venom PLA2 have favourable interactions with one other, which could result in inhibition (Alsamydai & Jaber, IJP, 2018).

9. Wound Healing

Wound healing is a multi-step process that involves inflammation, granulation, and tissue remodelling. Curcumin has been shown to improve wound healing in animals. Curcumin's wound healing impact is mediated by immuno-histochemistry localization of transforming growth factor-1, which demonstrated a rise in curcumin-treated wounds compared to untreated wounds, as well as collagen modulation and the reduction of reactive oxygen species. Curcumin also resulted in quicker re-epithelialization, enhanced neovascularization, greater migration of numerous cells into the wound bed, including dermal myofibroblasts, fibroblasts, and macrophages, and increased collagen content.

10.3 Conclusion

The experience and scientific knowledge of curcumin, a highly pleiotropic substance that has been employed in traditional medicine in many nations for its therapeutic properties. As indicated by the studies discussed above and the many more being reported every day, the pharmacological characteristics and applications of curcumin are a rapidly growing, progressing, and increasing enterprise. Despite its ethnobotanical importance, turmeric has been extensively researched for its bioactivity and therapeutic qualities. These are rich in bioresources, in terms of their substantial active principles or bio-active molecules (Fig. 10.2).

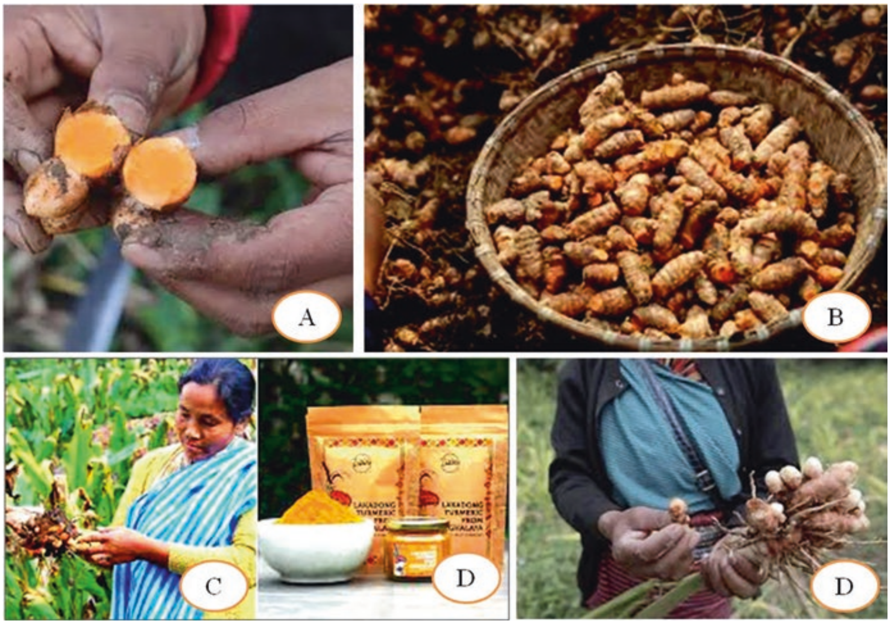


Fig. 10.2 (**a**) Lakadong finger, (**b**) Freshly harvested Lakadong finger, (**c**) Harvesting, (**d**) Packed turmeric Powder, (**e**) Fresh Rhizomes and Fingers of Lakadong. (Source:)

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Chapter 11
Micro- and Nanoparticle of Chitosan for Vitamin Encapsulation: A Nutshell Overview

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11.1 Introduction

Nanoscience and nanotechnology provide alternate approaches for producing functional diets, especially for the enrichment of bioactive substances without impairing the consumer's sensory experience. Thus, bioactive substances that are readily added to food and beverage products are distributed and protected using nanoencapsulation technology (Pateiro et al., 2021). These bioactive molecules include bioactive peptides, vitamins, antioxidants, probiotics, enzymes and proteins, all of which give physiological advantages or lower the chances of acquiring diseases over the long term. These bioactive substances are the subject of research studies (Sánchez & Vázquez, 2017). It is difficult to maintain the health-promoting properties of functional components since these molecules are volatile when they are processed (due to the variation in temperature, light and oxygen) or while they are in the stomach – facing changes in pH, enzymes and nutrition (Bell, 2002; de Souza Simões et al., 2017). These molecules may be preserved in diverse conditions via nanostructured systems such as liposomes, nanoemulsions, microemulsions, solid lipid nanoparticles and polymer nanoparticles. In food items, edible nanoparticles are used as a support or release system (McClements, 2018). Encapsulating several bioactive substances in these nanocarrier particles can improve their dispersibility, stability and bioavailability (Wang & Heuzey, 2016; Rafiee et al., 2019). The use of vitamin shows several difficulties due to high sensitivity and unstable nature under unsuitable environmental conditions (e.g. temperature, oxygen, light and humidity), and this leads to the search for new strategies for delivery. Thus, vitamins are described as small, low-molecular-weight organic compounds that are categorised into fat-soluble and water-soluble vitamins based on their solubility (Combs Jr & McClung, 2016; Abibu et al., 2019).

Nanoencapsulation is an alternative to maintain the strength of vitamins (de Britto et al., 2012; Luo et al., 2012; Giannakourou & Taoukis, 2021). The nanoencapsulation of vitamins contributes to the reduction of a lot of the abovementioned difficulties and results in increased stability and a longer shelf life (Fig. 11.1). In addition, they have applications of considerable interest, such as nutritional supplements for fortifying processed meals. Encapsulation is often used to cover the taste of vitamin- and mineral-enriched drinks in order to make them more palatable to customers (Chen & Wagner, 2004; Estevinho, 2022).

Nanocapsules, a class of nanoparticles, are encapsulated by a polymeric membrane and polymeric matrix containing the active chemical (Benita, 1998; Fang & Bhandari, 2010; Mora-Huertas et al., 2010; Xiao et al., 2022).

Choosing the suitable procedure for developing nanocapsules with the specified performance and functionality is also of utmost significance. The approach used will depend on the physicochemical properties of the polymer, the bioactive molecule encapsulated and the desired properties of the nanocapsule (surface area, shape, solubility, particle size and distribution, encapsulation efficiency and release procedure) (Pal et al., 2011; Rao & Geckeler, 2011; Ezhilarasi et al., 2013; Xiao et al., 2022). Given the polymer's physicochemical composition, numerous materials

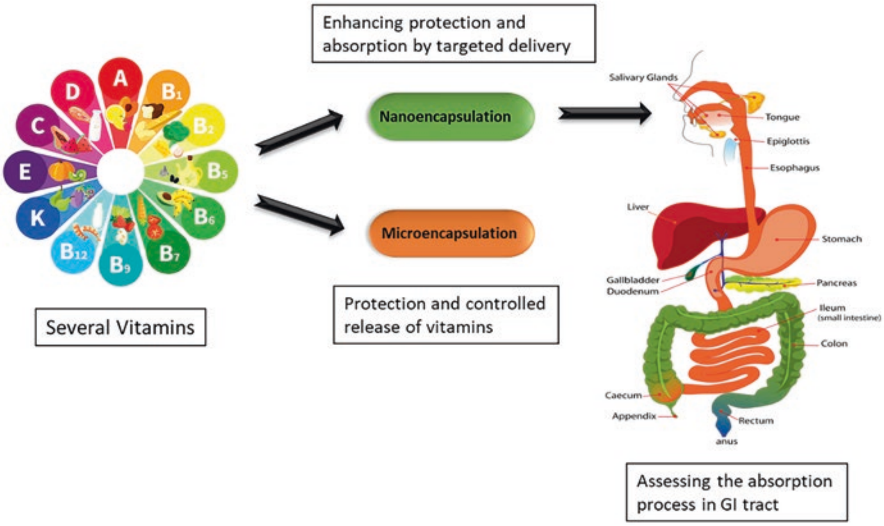

Fig. 11.1 Vitamin encapsulation for human health

have been employed in nanoencapsulation. Each encapsulation approach may be applied to every product or wall material (Ngwuluka et al., 2021). The polymer composition of bioactive chemicals (molecular weight, charge, nanosphere placement through adsorption or incorporation) and nanoparticles (surface charge, hydrophobicity and biodegradation profile) affects absorption, biodistribution and elimination (Reis et al., 2006; Herdiana et al., 2021). Polymeric colloidal nanoparticles produced from polysaccharides, lipids and biopolymers are also popular. The interaction of cationic and anionic biodegradable biopolymers creates polyanionic hydrogels with beneficial chemical entrapment and release characteristics. Chitosan is a well-studied biopolymer (Douglas & Tabrizian, 2005; Shahidi et al., 1999; Gajera et al., 2022).

A natural polymer called chitosan is often utilised to encapsulate and release active ingredients. A partially N-deacetylated derivative of chitin called chitosan is acid-soluble in comparison to chitin (Shahidi et al., 1999; Paul et al., 2019). Chitosan can be produced via the alkaline deacetylation of chitin from various crustacean species. The manufacture of chitosans with specific characteristics through fermentation with fungi is also being investigated (Rampino et al., 2013; Sebastian et al., 2020; Dmitrović et al., 2022). The monomers (glucosamine and N-acetylglucosamine) are joined together by b-1!4 bonds containing randomly acetylated amino groups (Şenel & McClure, 2004; Weißpflog et al., 2021; Reay et al., 2022). Chitosan has important qualities that are beneficial for a variety of applications and has appropriate availability for chemical reactions due to the amino and hydroxyl reactive functional groups (one primary and one secondary). On the other hand, chitosan's physical and chemical features, such as intramolecular and intermolecular hydrogen bonding and cationic charge in acid environments, make this polymer more appealing for the production of both traditional and innovative pharmaceutical products (Kas, 1997; Peter et al., 2021). New applications have been created as a consequence of the chemical derivatisation of chitosan properties. N-Acyl chitosans, which are utilised in the textile and medical sectors as well as the manufacture of membranes; N-carboxyalkyl chitosans, which are used in chromatography; and o-carboxyalkyl chitosans, which serve as molecular sieves, are some of these derivatives (Kumar, 2000; Bakshi, et al., 2020).

However, due to the biological and chemical characteristics of this polymer, the food sector has received much of the current commercial interest in chitosan (Aider, 2010; Bakshi et al., 2020). Its benefits include being derived from natural sources; being biodegradable, biocompatible and nontoxic; having polycationic qualities; being cheap cost to produce (Yenilmez et al., 2011; de Giglio et al., 2012; Ahmad et al., 2019); and having antibacterial capabilities (Pillai et al., 2009). Since this polysaccharide is a protection against unfavourable circumstances in the gastrointestinal tract, chitosan also functions as an encapsulating agent of active substances, boosting the absorption of the bioactive chemicals (Aranaz et al., 2009). The food and pharmaceutical industries are just beginning to explore nanoencapsulation-based technologies, which have benefits like enhanced bioavailability, high shelf stability and controlled release of active ingredients. Recent research on these techniques, advancements in the nanoencapsulation of vitamins, safety issues and health effects related to the consumption of these products are highlighted in this chapter. These findings open up new avenues for food technology and nutrition, with the potential for commercialisation.

11.2 Microencapsulation vs. Nanoencapsulation of Vitamins

Microencapsulation	Nanoencapsulation
Vitamins are protected from the environment	Greater surface area in relation to the mass proportion
Better flow characteristics	Formulating optically transparent vitamin solutions
Vitamins released gradually	Physical integrity bolstered against coalescence and gravitational separations
Determining the exact amount of vitamin delivery	Accelerated dissociation
Being affordable, particularly for the spray-drying process	High intracellular assimilation
Constructing vitamin solutions that scatter light	Make precision aiming possible
Add a multivitamin to the food items	Rendering long shelf-life-coated vitamins
Undesirable flavour of some vitamins is masked	A decrease in the amount of core-shell material used
	Reduce reactions between vitamins and surrounding molecules and media

11.3 Methods for Preparing Chitosan Micro- and Nanoparticles

Ohya et al. (1994) were the first to describe chitosan nanoparticles. They suggested that the anticancer drug 5-fluorouracil could be given intravenously using chitosan nanoparticles as a carrier system. These particles were made by emulsifying and gelating chitosan.

For many years, a lot of research has been done on these drug delivery systems. These formulations have also been taken into consideration for a variety of uses, including the addition of active ingredients to toothpaste (Liu et al., 2007; Mellou et al., 2019)). Additionally, these systems have been transformed by the use of several preparation techniques (Calvo et al., 1997; El-Shabouri, 2002). In addition, various researchers have discovered unique formulations of chitosan nanoparticles including components that generate secondary matrices (Sarmento et al., 2006; Grenha et al., 2010; Detsi et al., 2020).

Micro- and nanoparticles of chitosan have been produced using a different technique. Factors such as the particle size requirements, the environmental stability of the vitamin, the profile of the controlled release, the stability of the micro- or nano-encapsulated product and the potential residual toxicity of the final product influence the selection of the optimal approach. Coacervation, cross-linking, ionic gelation, emulsion coalescence technique, reverse micellar technique, sieving and spray-drying are among the most frequently employed techniques for the formation of chitosan micro- and nanoparticles (Agnihotri et al., 2004) (Table 11.1).

11.4 Cross-Linking

Covalent cross-linking chemicals such as formaldehyde (FA) and glutaraldehyde (GA) have been frequently used in the manufacture of chitosan microspheres. This process has various versions (Sinha et al., 2004). However, emulsion cross-linking

Table 11.1 Methods for chitosan micro- and nanoparticles for vitamin encapsulation

Method	Vitamin	References
Cross-linking	Carotenoids	Thamaket and Raviyan (2015)
Coacervation	Vitamin E	Alencastre et al. (2006)
	Lutein	Qv et al. (2011)
Spray-drying	Vitamin C	Desai and Park (2005)
	Vitamin D_2	Katouzian and Jafari (2016)
Ionic gelation	Vitamin C	Alishahiet al. (2011a, b)
	Vitamin E	Katouzian and Jafari (2016)
	Vitamin D_3	Luo et al. (2013)
Ionic gelation	Alpha lipoic acid	Velasco-Rodríguez et al. (2012)

is the most popular technique. A solution of chitosan in acetic acid is mixed with a liquid oil to form an oil-in-water (o/w) emulsion, to which a cross-linking agent is then added to produce microspheres, which are then filtered, washed and dried. This process is effective for encapsulating water-insoluble active substances, such as fat-soluble vitamins or phenolic compounds, that are spread with chitosan (Thamaket & Raviyan, 2015). The formation of chitosan nanocomposites with tetraethoxysilane has also been studied, although the encapsulation of any bioactive compound has not been attempted yet (Janes et al., 2001; Rosales-Martínez et al., 2018). Some alternatives have been documented, such as the water-in-oil emulsion solvent diffusion approach, which uses sodium tripolyphosphate (STPP) as a cross-linking agent, albeit with a different mechanism due to the ionic nature of the cross-linking.

11.5 Coacervation

The straightforward coacervation approach to making chitosan microbeads has some historical precedent. The coacervate droplets are created by dissolving chitosan in acetic acid and then introducing the solution through a nozzle into a solution of sodium hydroxide and methanol (Fig. 11.2). This technique has been used to immobilise enzymes, lipoproteins, cell cultures (Kumar, 2000; Rosales-Martínez et al., 2018) and some drugs (Gonçalves et al., 2005). Complex coacervation involves the addition of a polymer with an opposing charge. By this method using a carboxymethylcellulose-chitosan system, vitamin E has been encapsulated (Alencastre et al., 2006; Olusanya et al., 2022). These microbeads have a slightly non-Fickian releasing mechanism.

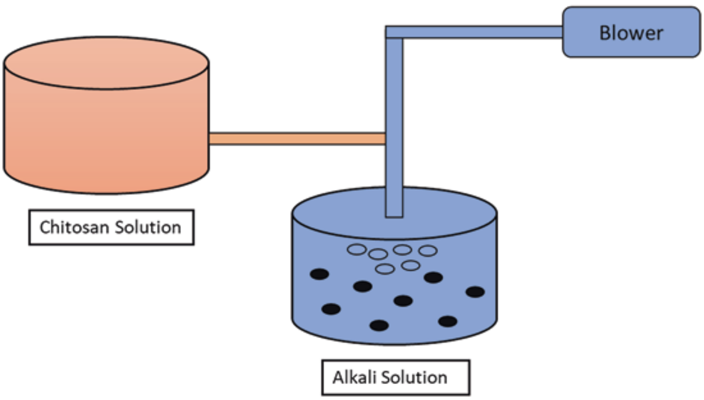

Fig. 11.2 Process of coacervation and precipitation

11.6 Spray-Drying

Since the 1950s, spray-drying microencapsulation has been employed in the food sector. Initially used to microencapsulate aroma components, it is now also utilised to microencapsulate probiotics and bioactive substances. Spray-drying is the preferred method at the industrial level for its relativity, speed and reproducibility, which facilitates the scale-up process compared to other micro- and nanoencapsulation technologies. The main factors to consider to optimise the microencapsulation process by spray-drying are the gas and liquid flow velocity and the gas inlet and outlet temperatures. Generally, and to give stability to the capsules, this method also makes use of some cross-linking agent. Inlet temperatures between 120 and 180 °C and suspension flow velocities between 0.12 and 0.42 L/h are the most typical process conditions.

Several features of the chitosan microcapsules must be governed by the parameters of the manufacturing procedure. The most significant aspects of the microencapsulated bioactive chemical are its size, shape, suspension stability and regulated release. According to Desai and Park (2005), the application of cross-linking agents such as STPP, GA or FA improves regulated release of bioactive chemicals and particle characteristics. They demonstrated that the capsules prepared with STPP had better swelling capacity, water uptake and release of the bioactive compound than those prepared with GA or FA. The same authors (Desai & Park, 2005) described the encapsulation of vitamin C in STPP cross-linked chitosan microspheres by spray-drying for oral delivery. They also showed that the release of the bioactive compound followed Fick's second law of diffusion. Oliveira et al. (2005) and Malekjani and Jafari (2021) used glyceraldehyde as a cross-linking agent, finding that although the properties of the capsules are not so good, the compound is not toxic as in the case of GA or FA. Other authors introduce variations in the process, as in the case of Kašpar et al. (2013) who use a three-fluid nozzle to generate microcapsules of chitosan and STPP with great stability. On the other hand, W. Liu et al. (2011) propose the use of a microfluidic aerosol nozzle followed by a microfluidic jet spray-drier to create stable microparticles of chitosan with homogeneous size and shape without the requirement for a cross-linking agent.

11.7 Chitosan Encapsulation of Vitamin C by Spray-Drying

Many foods include water-soluble vitamin C and ascorbic acid. Biology, pharmacy and dermatology utilise it to heal scurvy. It boosts immunity and decreases the risk of cancer, heart disease and excess lead (Pb) levels. Vitamin C must be consumed regularly via food since the body cannot create or store it. Citrus and green vegetables provide vitamin C. Microencapsulation may protect vitamin C from oxidative conditions. Spray-drying (Fig. 11.3) encapsulates vitamin C with the least ascorbic acid loss, and melt dispersion and thermal phase separation may release it. STPP

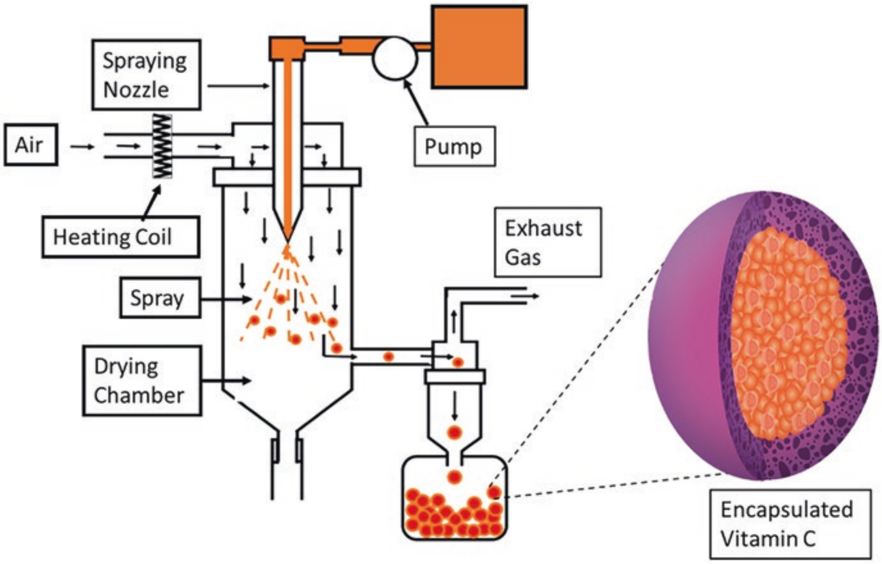

Fig. 11.3 Encapsulation of vitamin C with chitosan by spry-drying method

cross-linked a double-layered chitosan structure to control vitamin C release in gastric secretions and intestinal fluids. It was shown that when the cross-linking agent concentration was raised, the efficacy of encapsulation decreased; this may be due to the chitosan's surface flaws. Apply cross-linking agent in the specified amount at all times for improved control release and encapsulation efficacy.

11.8 Emulsion Coalescence Technique

This method was used to enhance the bioactive chemicals' diffusion and release from chitosan micro- or nanocapsules. The procedure entails the formation of two different forms of water-in-oil (w/o) emulsions. The first is made using chitosan that has been dissolved in an acetic acid or acetate buffer in a vegetable oil that also contains lecithin (Fig. 11.4). The second contains a 40 mM solution of copper sulphate that has been emulsified with lecithin in the same vegetable oil (Fig. 11.4). The second includes a 40 mM solution of copper sulphate emulsified in the same vegetable oil with lecithin. The second emulsion is added dropwise to the former with constant stirring. To accomplish the fusion of the two kinds of micelles and produce the chitosan microparticles, the resultant mixed emulsion was aggressively agitated (Tokumitsu et al., 1998; Kofuji et al., 2005). This technique has also been successful when microfluidisation was used for the fusing process (Mazutis & Griffiths, 2012). Other variants that do not involve coalescence such as the w/o emulsion solvent diffusion method, using ethyl acetate as the oil phase and STPP as

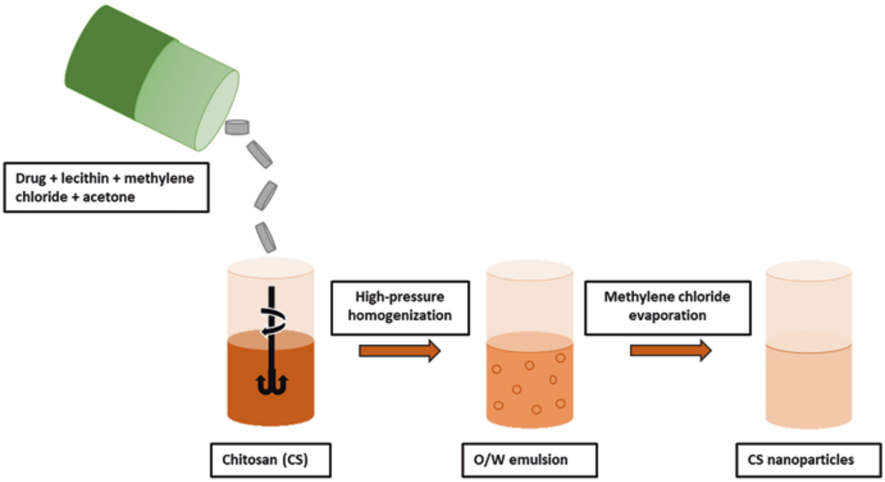

Fig. 11.4 Representation of emulsion solvent diffusion

the cross-linking agent, have also been studied given the simplicity of their preparation (Mikušová & Mikuš, 2021). Similar procedures have been used to prepare chitosan magnetic microspheres (Denkbaş et al., 2002; Geng et al., 2012).

11.9 Ionic Gelation

On the basis of the spontaneous complex formation between the polysaccharide chitosan and various polyanions, many methods for producing chitosan nanoparticles have been suggested (Calvo et al., 1997). In particular, chitosan nanoparticles prepared by ionic gelation have been of great interest, since it is a simple and smooth process, nontoxic, free of organic solvents, controllable and used as a means of administration for low-molecular-weight drugs (Janes & Alonso, 2003; Fan et al., 2012; Cota-Arriola et al., 2013). Sinha et al. (2004) mention that polyanions for ionic gelation, also known as ionotropic materials, can be divided into two categories: (a) hydro-phobic polyanions (HPP), such as sodium al (e.g. octyl sulphate, dodecyl sulphate, hexadecyl sulphate and acetyl stearyl sulphate), and (b) low-molecular-weight (LPW) polyanions (such as sodium pyrophosphate [SPP], tripolyphosphate, tetrapolyphosphate, octapolyphosphate and hexametaphosphate). Since STPP is nontoxic, multivalent and capable of forming gels via ionic cross-linking interactions, it has often been utilised to make chitosan nanoparticles (Fig. 11.5). The interaction can be controlled by the charge density of STPP and chitosan, which depends on the pH of the solution (Zhao et al., 2011; Rosales-Martínez et al., 2018). In this respect, it is well known that, depending on the mechanism used, cross-linking may be accomplished in acidic, neutral or basic conditions (Table 11.2).

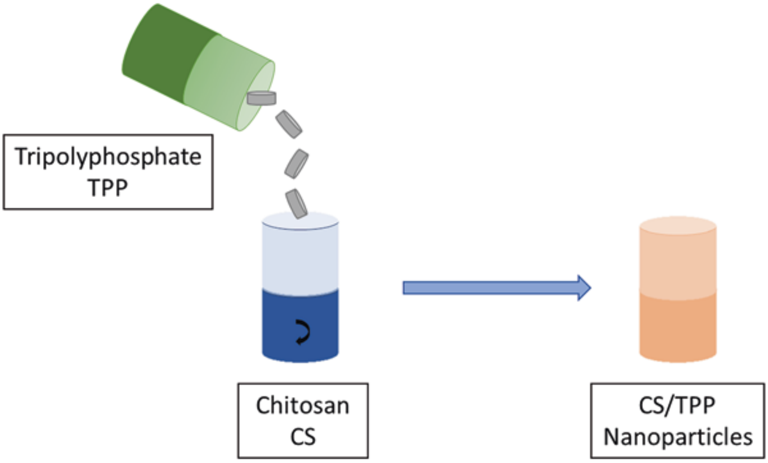

Fig. 11.5 Representation of ionic gelation method

Table 11.2 Different approaches along with advantages and disadvantages

Methods	Advantages	Disadvantages	References
Cross-linking	Better encapsulating efficiency Improved stability Applicable for large scale	Low thermal stability Fail to clinical trial to reach the target site	Katouzian and Jafari (2016) and Raza et al. (2020)
Coacervation	Effective as a temperature-sensitive active encapsulate Utilisation of organic solvents In large scale, it is suitable On the surface of microcapsules, coacervating materials are present	Complex procedure Complex coacervates have low stability The use of hazardous chemicals in the procedure Expensive method	Ma et al. (2019) and Timilsena et al. (2019)
Spray-drying	Effective encapsulation of protected product Economical Industrial use User-friendly	Particle size management is difficult High-temperature sensitivity Minimum yield for small batches	Raza et al. (2020) and Sarabandi et al. (2020)
Emulsion	The microscopic size of microcapsules Living cells can be encased It is possible to encapsulate both hydrophobic and hydrophilic active ingredients	Thermal stability is lower Selected emulsifiers are used	Merkl et al. (n.d.) and Sarabandi et al. (2020)
Ionic gelation	The method is very economic and simple The method requires less equipment and time No use of organic solvent	TPP/CS nanoparticles are their poor mechanical strength Possible particle disintegration is due to the weakness of the ionic interactions	Raza et al. (2020)

11.10 Effect of Various Parameters on the Physicochemical Properties of Nanoparticles

Numerous scholars have researched the effects of various elements, including the molecular weight and concentration of chitosan; the concentration of polyanion STPP; and the pH, type and concentration of the bioactive molecule to be encapsulated, among others (Hans & Lowman, 2002; Amidi et al., 2006; Triwulandari et al., 2018). Tang et al. (2007) studied the effect of factors such as molecular weight and chitosan concentration, STPP concentration and pH of the solution in the size of chitosan nanoparticles used to immobilise a proteolytic enzyme. As a result, they observed a gradual increase in particle size by increasing the molecular weight of chitosan, attributing this to the fact that the higher-molecular-weight chitosan interacted with STPP more efficiently than lower-molecular-weight chitosan. Additionally, the higher-molecular-weight chitosan is less soluble, and as a consequence, an increase in particle diameter was obtained. The minimum particle size obtained was 42 ± 5 nm under optimal immobilisation conditions (1 mg of neutral proteinase immobilised for 15 min at 40 °C, resulting in an enzymatic activity yield of 84.3%). Yang and Hon's (2009) similar findings were achieved when nanoparticles of 70.6 nm were prepared using chitosan with a molecular weight of 55 kDa.

Accordingly, Gan and Wang (2007) produced chitosan-STPP nanoparticles by altering the parameters evaluated by the aforementioned author. They noticed a straightforward linear connection between the fraction of chitosan-STPP and the particle's size and zeta potential. In addition, they exhibited a substantial positive surface charge throughout a wide pH range below the isoelectric point of 9, which was mostly due to the presence of positively charged amino groups. The pH value of the solution and the chitosan concentration also influenced the stability of the nanoparticle system, and the chitosan concentrations for the spontaneous formation of particle aggregates were 0.65%, 0.25% and 0.15% (w/v) at pH 4 and 1.00%, 0.85% and 0.75% (w/v) at pH 5.0 for low-, medium- and high-molecular-weight chitosan, respectively. Since chitosan can only be dissolved or dispersed at acidic pH levels, the molecule has undergone chemical modifications. One such modification is the quaternisation reaction, which improves the aforementioned polymer's solubility over a wide pH range and allows for greater control over its cationic properties. Additionally, it has been noted that the STPP load falls below 3 at pH 4, which reduces its cross-linking ability; however, at pH >6, the charge density and strength of the electrical interactions decrease, which also reduces the STPP's solubility in water (Guo et al., 2007; Layek & Nandi, 2013). Nasti et al. (2009) showed that transient exposure of nanoparticles to a higher pH may induce some chitosan aggregation, increasing the number of nuclei available for nanoparticle growth (at higher core velocity, smaller particles will be present). They obtained nanoparticles with a size of 200 nm, which were then coated with hyaluronic acid (HA), and nanoparticles with a size of 200–400 nm, which were utilised as a control for HA-coated nanoparticles with comparable dimensions but distinct surface characteristics.

The link between the free amino groups on the surface and the properties of the chitosan nanoparticles produced by ionic gelation is an additional crucial factor (Szymańska & Winnicka, 2015; Esquivel et al., 2015). Lin et al. (2007) reported that by increasing the pH in chitosan nanoparticles, free amino groups can be deprotonated, thereby decreasing particle size and zeta potential. On the other hand, they observed that the amount and level of ionisation of the free amino groups on the surface are affected by the degree of gelation and the pH. As for the release of bioactive compounds, this type of nanoparticle presents difficulties in releasing large quantities of relatively large molecules such as bovine serum albumin (BSA) (Gan & Wang, 2007). On the other hand, Xu and Du (2003) observed that the rate of release of BSA slightly increased if the degree of deacetylation was decreased from 92% to 75.5%. Additionally, it has been discovered that the rate and extent of active chemical release rise in acidic medium and decrease in neutral or basic media (Lin et al., 2007; Rosales-Martínez et al., 2018).

Regarding the effect of the amount of polyanion on the particles, Bao et al. (2008) claim that excessive STPP increases electrostatic interactions, which adds to the rise in particle size.

11.11 Use of Other Polyanions

Avadi et al. (2010) used gum Arabic as a polyanion with good results to encapsulate insulin in chitosan. It has also been described that polyethylene oxide and negatively charged cyclodextrin have been used as polyanions to encapsulate various bioactive compounds. Racoviţă et al. (2009), Wani et al. (2016), and Aral and Akbuğa (1998) prepared chitosan microbeads with diameters between 0.78 mm and 0.92 mm using sodium alginate as the polyanion and STPP as the cross-linking agent to encapsulate BSA. To obtain nanosized particles, Gupta and Karar (2011) optimised the process variables (chitosan concentration, speed and agitation time and amount of bioactive compound to be encapsulated) for their preparation. They were able to obtain nanoparticles with diameters between 230 and 627 nm and maximum bioactive compound loading capacity.

11.12 Reverse Micellar Process

Reverse micelles are nanodroplets generated from thermodynamically stable aqueous-oil-surfactant liquid mixtures (Uskokovi & Drofenik, 2005; Hegde et al., 2013). This process permits the creation of nanoparticles with diameters less than 200 nm and low polydispersity indices, which is a benefit. This approach was employed by Mitra et al. (2001) using n-hexane as the oily phase and GA as the cross-linking agent.

11.13 Desolvation

Dripping a sodium sulphate solution into a suspension of chitosan with Tween 80™ stabiliser under agitation and/or sonication results in the desolvation of chitosan into 900 nm diameter nanoparticles that may adsorb hydrophilic molecules on their surface (Janes & Alonso, 2003; Hegde et al., 2013). All that has been described up to this point comprises bottom-up manufacturing processes, which involve the assembly of molecules into a solution to form, defined in this case, micro- and nanoparticles (Chan & Kwok, 2011; Biswas et al., 2012).

11.14 Sieving Technique

In this scenario, the bioactive chemical is first produced, and then a mass of chitosan gel is created and cross-linked with GA. This mixture is run through sieves with pores that are appropriate for the desired microparticle diameter (often between 500 and 600 μm). The particles have a high PI yet exhibit excellent bioactive chemical diffusion (Agnihotri et al., 2004; Barhoum et al., 2019).

11.15 Different Nanocarriers Developed with Chitosan

Nanostructures are materials with novel physical and chemical properties and a nanoscale size in at least one dimension of 1–1000 nm; usually, they are defined by a diameter of 1–100 nm. There are several varieties, including nanoparticles, nanoclusters, nanotubes, films and nanocomposites. Changes in nanostructures' surface-to-volume ratio and electrical characteristics considerably enhance their functional and industrial applications. The goal of food scientists is to create specialised nanostructures generated from foods while maintaining their original origin. Due to their functional groups with a wide range of binding and trapping potentials, polysaccharides like chitosan are frequently used as dietary components. Figure 11.6 shows a schematic overview of chitosan-based nanostructures and associated production techniques.

11.16 Chitosan Particles as Stabilisers in Pickering Emulsions

Solid particles stabilise Pickering emulsions, which are systems of minute droplets distributed from one immiscible liquid into another. Many of these systems include particles containing irritating surfactants that cannot be utilised in food. The use of

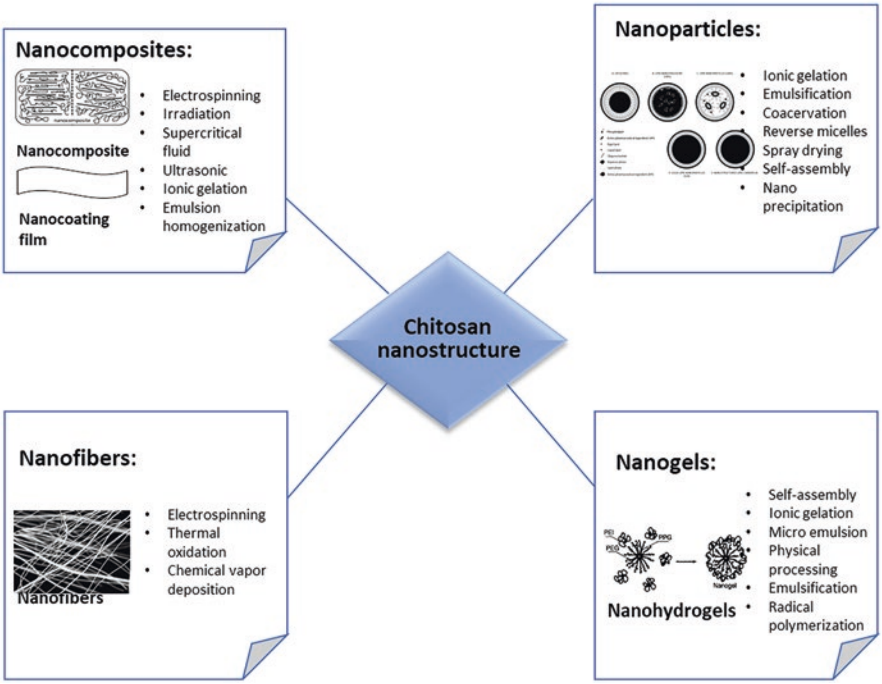

Fig. 11.6 Chitosan nanostructures and their method of preparation

polysaccharides such as starch and chitosan is a suitable alternative for the food industry. Among the early experiments are those of Zhang et al. (2015), who prepared stable liquid paraffin-in-water Pickering emulsions using a mixture of polystyrene nanoparticles with 90% deacetylated chitosan. Emulsions were prepared by ultrasound application, and the preponderant role of chitosan was observed in the stabilisation. Wang and Heuzey (2016) studied the possible use of chitosan only as stabilising and emulsifying agent whose properties depend on pH. They prepared, using ultrasound processing, corn oil in water emulsions at different pH values using 1% chitosan with 90% deacetylation as a stabiliser. They observed that at pH values from 3.5 to 5.5, conventional o/w emulsions, with decreasing drop diameters ranging from 14 to 2.1 μm and maximum stability of two months, were formed. When pH was adjusted to 6.5, the chitosan molecules self-assembled into 82.1 nm nanoparticles and formed Pickering emulsions with a droplet diameter of 1.7 μm and stabilities of up to five months. On the other hand, Shah et al. (2016) prepared chitosan-STPP nanoparticles by ionic gelation and used them to stabilise Pickering emulsions in which they encapsulated polyphenolic curcumin, a powerful anticancer and antioxidant compound. The ratios of the components produced droplets with diameters ranging from 40 to 200 μm, a homogeneous distribution of particle size and minimum stabilities of 30 days.

11.17 Chitosan Particles as Vitamin Carrier Systems

In addition to the previously mentioned criteria, the effect of adding bioactive chemicals, such as vitamins, to chitosan-based nanoparticulate systems is of major interest. De Britto et al. (2012) investigated the influence of vitamin C supplementation on chitosan nanoparticles and reported a considerable increase in particle size (196 ± 8 at 534 ± 20 nm). This was also observed in the case of folic acid (vitamin B9) and cobalamin (vitamin B12). In the first case, they linked it to the vitamin's production sequence and charge density. Due to the presence of acid and carbonyl groups, vitamin C (L-ascorbic acid) has a high density of negative charges despite its low-molecular-weight and simple structure. When the vitamin was first introduced to the chitosan solution, electrostatic interactions (mostly ionic and hydrogen bonding) were started between these two oppositely charged molecules (Alishahi et al., 2011a, b).

Applying STPP and the ionic gelation method, Alishahi et al. (2011a, b) produced particles using chitosans of various molecular weights to encapsulate vitamin C, resulting in particle sizes ranging from 185.4 ± 2.1 to 585.3 ± 3.6 nm for chitosan's with molecular weights between 65 and 450 kDa. It was also demonstrated that the useful life and release of vitamin C increased. Vitamin C was more effectively encapsulated by low-molecular-weight chitosan, and its release from nanoparticles was pH-dependent. In a fish trial, these capsules allowed for a controlled in vivo release of the vitamin for up to 48 h (Alishahi et al., 2011a, b). It has also been claimed to encapsulate vitamin C as ascorbyl palmitate using the o/w emulsion approach, with STPP acting as a cross-linking agent. In this case, the charged particles had sizes ranging from 30 to 100 nm and a good vitamin release at pH 8.0 (Yoksan et al., 2010; Caritá et al., 2020).

Regarding the effect of the addition of vitamin B9, de Britto et al. (2012) found a significant increase in the size of the nanoparticle. Vitamin B9 is essentially insoluble in an acid medium and, under certain circumstances, is chemically favoured by being in the solid phase (precipitate of nanoparticles) as opposed to the liquid phase (chitosan acid solvent). Similarly, the influence of particle size on vitamin C- and E-containing systems is discussed. Since these vitamins are highly soluble in an acid media, it is advantageous for them to reside in the liquid phase; hence, the increase in nanoparticle size was not substantial. In terms of zeta potential values, vitamin B9 showed an increase (64.9 ± 4.6 mV), but vitamins C and E showed a reduction (30.7 ± 3.7 mV and 29.4 ± 3.1 mV, respectively). Lastly, it was shown that the success of encapsulation is dependent on the structure of the nanoparticles and the solubility of the vitamin, with vitamin B9 being the most effectively encapsulated (about 40%). On the other hand, Naghibzadeh et al. (2010) obtained chitosan particles loaded with tocopherol and STPP, with particle sizes ranging between 277 and 378 nm. The zeta potential values of the nanoparticles were greater than +30 mV, which indicates adequate stability. Weerakody et al. (2008) prepared chitosan microspheres loaded with a-lipoic acid by spray-drying without the addition of cross-linking agents. They obtained microspheres of 7.89 μm in diameter and an encapsulation efficiency of 55.2%. Velasco-Rodríguez et al. (2012) prepared chitosan nanoparticles (high and low molecular weight) loaded with a-lipoic acid using SPP and STPP as polyanions, obtaining particle sizes between 180 ± 74.6 nm and 489 ± 25 nm. An encapsulation efficiency of 62.4% was achieved by using STPP as polyanion. Except for those synthesised with STPP and the stabiliser poloxamer 188, all chitosan nanoparticles were stable at pH level 5.3, suggesting a promising use in meals. Using a combination of n-hexane, n-hexanol and surfactant as the oil phase, Milašinović et al. (2016) used a reverse emulsion method to obtain chitosan microspheres loaded with a-lipoic acid using a mixture of n-hexane, n-hexanol and surfactant as the oil phase. Microspheres with diameters between 270 and 660 m, encapsulation efficiencies between 46.8% and 58.5%, acceptable antioxidant activity retention and enough sustained release were produced.

Aresta et al. (2013) reported the applicability of vitamin-added chitosan nanoparticles in novel food packaging. Ionic gelation was used to produce nanoparticles of chitosan enriched with vitamins C and E. The nanoparticles formed were measured for size and zeta potential, yielding a size range of 375–503 nm and zeta potential values between +16.0 and + 33.8 mV after the addition of these vitamins. Finally, Azevedo et al. (2014) studied the encapsulation and controlled release of vitamin B2 inside alginate and chitosan nanoparticles. The size of the alginate/chitosan nanoparticles decreased from 119.5 ± 49.0 nm to 104.0 ± 67.2 nm. This resulted in PDI values of 0.4540.066 for samples devoid of vitamin B2 and 0.3190 ± 0.068 for those with vitamin B2. Similarly, they achieved an encapsulation efficiency of 55.9 ± 5.6% and a load capacity of 2.2 ± 0.6%. The evaluation of vitamin B2 release profiles under different conditions found that polymer relaxing is the most relevant component.

In an alternative use, chitosan was used to coat 300 nm nanoparticles of zein loaded with retinol. After coating, an increase in size up to 500 nm was observed, the zeta potential increased from −30 to +24 mV and the encapsulation efficiency rose from 64.9% to 80%. Likewise, the release rate decreased and retinol protection against ultraviolet radiation increased. All of this indicates that it is possible to modify the properties of nanoparticles of a protein nature using a coating with a charged compound such as chitosan (Park et al., 2015).

Vitamin release from micro- and nanocapsules depends on their cross-linking, shape, size, density and vitamin physicochemical characteristics (Agnihotri et al., 2004; Maleki et al., 2022). Fourier transform infrared spectroscopy measurements reveal that the vitamins present in STPP cross-linked chitosan microspheres are stable. By Fick's second rule of diffusion, the release of vitamins from chitosan capsules is maintained and largely governed by the degree of cross-linking (Desai & Park, 2005). Due to its resistance to gastric degradation, using chitosan as a wall material for micro- and nanoencapsulation is desirable. Gut microorganisms, on the other hand, may effectively degrade it, releasing the vitamin for absorption. Additionally, chitosan has mucoadhesive qualities (Agnihotri et al., 2004; Maleki et al., 2022).

11.18 Challenges and Future Prospects

Stability of nanoparticles is dependent on several factors, including pH, ions and specific enzymes. Nanoparticles of chitosan containing vitamin C may encounter a variety of pH conditions, unique enzymes and excessive ions as they enter the digestive system, which might affect vitamin C absorption. As vitamin C-containing chitosan nanoparticles enter the digestive tract, they may face a variety of enzymes, pH conditions and excessive concentrations of ions, which may impact vitamin C absorption. The reaction of chitosan nanoparticles with specific enzymes in the GI tract may alter the nanoparticles' characteristics.

The main cause of colour and quality changes is the breakdown of vitamin C during food preparation and storage. Thus, the most sustainable form of vitamin C is required for nutrition of human and animal. To increase vitamin C's stability and prevent oxidation or degradation while delivering it to a specified location, encapsulation and controlled release are viable techniques. Chitosan particles have been shown to protect active substances from the hostile environment of the gastrointestinal system, increasing absorption.

Bioavailability and permeability of active pharmaceutical ingredients (APIs) are critical drug delivery parameters. Several in vitro, in vivo and clinical trial strategies have been developed and evaluated to alter these two characteristics. Chitosan nanoparticles have been extensively used as delivery vehicles for bioactive compounds and active pharmaceutical ingredients (APIs), including proteins, peptides, micronutrients, plasmids, medicines and polynucleotides. Chitosan nanoparticles may enhance medication retention capacity, pharmacokinetic bioavailability, adhesion and prolonged release of active components in vivo and during storage. Therefore, chitosan may be used to actively provide vitamin. To discover well-coordinated chitosan nanoparticles for vitamin C encapsulation, more study is required. Bioavailability and targeting may be enhanced by modifying chitosan nanoparticles. By altering the hydroxyl and amine groups in chitosan, the material's physical characteristics may be enhanced. Chemical alteration may enhance temperature stability, pH sensitivity and targeting accuracy. Before proceeding with in vivo studies, it is necessary to establish the toxicity of newly created nanoparticle systems.

11.19 Conclusions

Chitosan is a polymer of tremendous importance as a material for the micro- and nanoencapsulation of important bioactive components in foods, but no commercial use has yet been found. Methods for preparing micro- and nanocapsules vary depending on, among other factors, the material to be encapsulated, the molecular weight of chitosan, the cross-linking agent, the food into which the capsules will be inserted and the desired release rate. All of the aforementioned factors will make it possible to create superior functional foods with encapsulated bioactive components, such as vitamins, that are preserved and released at the right place.

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Chapter 12
Nanofertilizers: A Futuristic Approach to Crop Production and Towards a Sustainable Environment

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12.1 Introduction

Providing food security is one of the most serious issues in the modern world. In this regard, conventional farming practices must be improved by incorporating new and developing approaches that are both quick and cost-effective. Nowadays, nanotechnology is one of the most important research and development tactics that has consistently assisted mankind in flourishing, even in the face of adversity. Nanotechnology has the potential to contribute significantly towards the upliftment of the agricultural sector, and several research experiments have been done in this regard in recent years (Ditta, 2012). To address global challenges such as a rapidly expanding human population, changing climatic conditions and decreasing accessibility of critical plant macronutrients and other nutrients, agricultural yields should be increased by utilizing a variety of modern approaches. Nanotechnology has also shown the potential in augmenting global food production, improving food nutritional content and reducing leftovers for the "sustainable intensification" of agricultural output (Mousavi & Rezaei, 2011; Pérez-de-Luque, 2017; Bhardwaj et al., 2022). The widespread use of chemicals in the form of pesticides and fertilizers is the leading source of environmental pollution and the extinction of biodiversity in each ecosystem. Overcoming the drawbacks, several nano-based formulations provide channels for fertilizer delivery that are both effective and target-specific, resulting in increased yield and reduced damage. Hence, food security and safety are the main emphasis areas of nanotechnology research in the agricultural aspects which may lead to achieving sustainable crop production goals.

12.2 Nanotechnology: An Approach to Sustainable Agricultural Development

Nanotechnology is considered a novel science that deals with the study of production, development, manipulation and use of nanometric scale materials with dimensions ranging between 1 and 100 nm (Thakkar et al., 2010). Nanomaterials vary from their existing materials and are expected to change their physicochemical characteristics, gaining remarkable qualities, functionalities and high reactivity as a result of the more surface area-to-volume ratio of the particles (Nair et al., 2010). Nanotechnology is considered an outstanding technology with numerous potential applications in recent decades (Marchiol et al., 2019).

Nanoparticle engineering is a recent technological advancement that demonstrates distinct targeted properties with increased potential. The term "nanotechnology" was first used in 1974 by Professor Norio Taniguchi of Tokyo University of Science (Khan & Rizvi, 2014). Even though the word "nanotechnology" has been around for a while in a number of fields, the use of nanoparticles (NPs) in the agriculture sector is relatively new. Nanomaterials of various diameters offer multiple uses in medical science, environmental study, agriculture and food processing sectors. There are three main categories of nanoparticles: naturally occurring, accidental and manufactured. Viruses, mineral mixtures and sea spray are a few examples of naturally formed nanoparticles. Incidental nanoparticles have been synthesized by human-made industrial activities, such as sandblasting, welding gases, diesel exhaust, kitchen smoke and industrial effluents. All artificial particles with nanoscale size are considered engineered nanoparticles. Metals, nanotubes, quantum dots, sunscreen pigments and nanocapsules are some examples.

Furthermore, agriculture faces a broad spectrum of new and unforeseeable issues, the majority of which are associated with a decrease in crop yield due to several stress factors (biotic and abiotic), nutrient insufficiency and degradation of soil health. Nanotechnology has emerged as a possible solution to these issues. Modern agriculture emphasizes precision farming which is the use of wireless connections and sensor miniaturization to monitor, evaluate and regulate agricultural activities. Precision farming more specifically deals with site-specific management for nutrients and pest infestations covering diversified crops extending from horticultural crops to field crops. By using nanoscale nutrients to boost uptake or by adding nano-carriers for the efficient transport of active components to reduce losses while increasing yields through better nutrient use efficiency, nanotechnology opens more possibilities for precision farming (Gogos et al., 2012; Martínez-Fernandez et al., 2016). Nanotechnology also offers outstanding answers to critical environmental issues. For example, the development of nanosensors offers a lot of potential for monitoring environmental stress and improving plants' disease-fighting abilities. Therefore, further advancements in nanotechnology with a focus on recognizing constraints and implementing a collaborative framework for sustainable agricultural growth have the potential to offer important societal and equitable benefits. It's seen to be one of the solutions to the immediate concerns of sustainable production and food security, resulting in lower production costs and higher yields.

12.3 Role of Nanofertilizer in Agriculture

Nanofertilizers are nanometer-scale compounds containing major and micronutrients that are applied to crops in a regulated manner (Adisa et al., 2019; Shang et al., 2019). Chemicals can be treated using nanotechnologies on their own or in combination with several other ingredients in agricultural goods. Nutrients can also be given as nanoscale particles or emulsions, or they can be enclosed within nanoporous materials (Rai et al., 2012). Due to their ability to be administered in smaller amounts than conventional fertilizers, nanofertilizers have the potential to significantly contribute to environmental conservation by lowering runoff, leaching, reducing soil toxicity and gas emissions into the atmosphere (Naderi & Danesh-Shahraki, 2013; Manjunatha et al., 2016; Adisa et al., 2019). The few organic nanomaterials used for nano-encapsulation to enhance the efficacy, distribution and accessibility of nutrients and agricultural inputs are liposomes, chitosan and dendrimers (Anu Puri et al., 2009). Nanoparticles have been synthesized using semiconductors, polymers of both synthetic and natural origin, oxides of metals, ceramics, magnetic materials, lipids and emulsions (Kumar et al., 2018; Ruiz-Cañas et al., 2020). Clays, zeolites, zinc oxide (ZnO), silicon oxide (SiO_2) and titanium oxide (TiO_2) are among the inorganic nanoparticles used in this smart delivery system (Chinnamuthu & Boopathi, 2009; Zhao et al., 2014). Currently, a variety of products like nano fungicides, bactericides, growth regulators and fertilizers have been developed using nanotechnology applications with the potential to use as agrochemicals (Peters et al., 2014; Pestovsky & Martínez-Antonio, 2017).

12.4 Need and Possible Benefits of Nanofertilizers upon Conventional Fertilizers

Several causes have accelerated research on NFs, including poor fertilizer-to-response ratios, the appearance of multi-micronutrient deficits in most agricultural soils due to imbalanced fertilization and the threat of environmental deterioration. Nanofertilizers have ample benefits over conventional fertilizers, such as the following:

(i) *Slow and Controlled Release of Nutrients Through Nanomaterials*

The nutrients from conventional fertilizers may be released to the ecosystem in several ways such as the (a) volatilization of applied nitrogen in form of gaseous ammonia and nitrous oxides and (b) chemical precipitation of phosphatic fertilizers by forming calcium, iron and aluminium phosphates, leaching loss of nitrates or phosphates and microbial conversion of inorganic nutrients to organic form. The regulated and slow release of micronutrients, biofertilizers and fertilizers for maximum effectiveness is one of the prospective applications of nanotechnology in agricultural research. Fertilizers can discharge slowly with the aid of nanomaterials. Surface coatings or nanocoatings of nanomaterials on fertilizer particles effectively keep the substance away from the plant due to higher surface tension than on conventional surfaces (DeRosa et al., 2010).

(ii) *Higher Nutrient Use Efficiency (NUE)*

The major drawback of conventional fertilizers is their low-nutrient use efficiency. Nitrogen, phosphorus and potassium have the lowest utilization efficiency of all the main macronutrients. If not used by the plant, approximately 40–70% N, 80–90% P and 50–70% K supplied through conventional fertilizers are lost as leaching and volatilization and leaching process and are flushed to surface water sources to end up causing eutrophication or converted into recalcitrant soil organic matter by soil microflora activity (Monreal et al., 1986). Surprisingly, this leaves only a small portion of the applied fertilizers (30–60% N, 10–20% P and 30–50% K) accessible for plant uptake. The use of NFs is anticipated to effectively avoid the low nutrient usage efficiency of traditional fertilizers, increase plant development and increase yield while using far lower dosages of the same nutrient than would be required if it were provided in bulk form (Herrera et al., 2016).

(iii) *Biofortified Crops Through NFs*

Additional advantages of employing nanofertilizers include improved nutritional quality (biofortification), tolerance to a variety of biotic and abiotic stress conditions and the utilization of nano-based hydrogels and other polymeric substances as powerful soil conditioners.

The World Health Organization has designated nanomaterials (NMs) containing micronutrients like iron, zinc and selenium as necessary dietary elements for animal and human growth and development. The nutritional content of food can be improved by priming, coatings or fortifying these minerals in nanoform (Subbaiah et al., 2016). Siva and Benita (2016) reported that a significant increase in the iron content of ginger plants was obtained when ferric oxide nanoparticles were applied. Furthermore, Morales-Diaz et al. (2017) revealed that plants with superior nutritional content and physiological functions have a better ability to adapt to stressful environments.

(iv) *Ecological Benefits*

The environmental benefits of nanotechnology can include the reuse and recycling of biowaste generated in agricultural practices such as husk, stover, straw, pod covers and other materials that can then be used to build nanoscale products with the mechanism of nanodelivery for nutrient elements. The severe environmental consequences of burning or dumping rice husks and straws can be minimized by transforming field biowastes into nanosilicate particles, which can be used for the adsorption and sequestration of nutrients (Wanyika et al., 2012). The nanosilicate, which is made from rice husk, could also be used to remove arsenic from the ground as well as surface water bodies.

Further, the NM-based composites or polymeric substances will not only ensure soil particle binding to prevent erosion, but the polymer's swelling potential will also aid in absorbing and storing large amounts of water, as well as decontamination of pesticide residues, heavy metals and radionuclides that are harmful to plants, animals and humans (Majeed & Taha, 2013).

12.5 Salient Features of Nanofertilizer

Nanofertilizers have the following features that make them beneficial and favourable over regular fertilizers:

(a) *Higher surface area*: Nanofertilizers have a large diameter due to their small size of particles, which helps them to be delivered to different sites in the plant system to aid various metabolic processes, resulting in the formation of higher photosynthates. As a result of the greater surface area, the nutrient uptake and use efficiency increase by increasing the reactivity of nanofertilizers with other compounds.

(b) *Smaller size of particles*: Nanofertilizers have a particle size smaller than 100 nano-meters, resulting in an increase in their absorption capacity by plant cells from applied substrates (soil or leaves) (Liscano et al., 2000).

(c) *High solubility*: High solubility of nanofertilizers in a variety of solvents is a special feature that aids in the dissolution and dispersion of insoluble nutrients in the soil, resulting in enhanced nutritional bioavailability.

(d) *High penetration capacity and controlled release of fertilizers*: Nanofertilizers serve a vital part in increasing a plant's nutrient availability and consequently promote seedling growth due to their high rate of penetration and regulated release of nutrients. The controlled release of nanofertilizers reduces fertilizer toxicity. As compared to bulk $ZnSO_4$, nano-ZnO had greater germination and root growth rate in peanut seeds (Prasad et al., 2012). Zeolite-based nanofertilizers boost nutrient availability to crops throughout the growth cycle; reduce nutrient loss through denitrification, volatilization and leaching; and fix nutrients in the soil, particularly NO_3 and NH_4.

(e) *Enhanced nutrient use efficiency*: The use efficiency of nutrients and nutrient uptake ratios in crop production can be enhanced through nanofertilizers. Bulk fertilizers have short-term effectiveness; however, nanofertilizers can extend the period of nutrient delivery (Cui et al., 2010).

12.6 Synthesis of Nanomaterial

A variety of techniques, including physical, chemical, physicochemical (aerosol) and biological methods, can be used to create nanoparticles. Nanoparticle production approaches usually used either a top-down or bottom-up approach. Chemical methodologies for manufacturing nanomaterials are the conversion of elemental structures into larger stable structures known as bottom-up methods. Self-assembly and positional assembly are methods for generating ultrafine-sized particles from their dissolved molecular state utilizing suitable solvents. Bottom-up approaches are commonly used to synthesize nanoparticles for plant applications (Saha & Gupta, 2017).

The conversion of bulk-sized materials to nanosized particles through crushing, powdering or grinding is accomplished in the physical approach (top-down approach) of nanoparticle synthesis (De Castro & Mitchell, 2002). The most used top-down method is powerful ball milling, previously known as mechanical milling. This method synthesized nano-scaled particles with a larger surface area by breaking down large-sized particles via drastic plastic deformation which increases the reactivity of the particles. Major techniques used in the synthesis of nanoparticles (NPs) are listed in Table 12.1 along with a comparison between top-down and bottom-up approaches.

Table 12.1 Comparison between two major approaches to the production of nanomaterial

Approaches	Advantages	Limitations	References
Bottom-up approach Major techniques: Plasma arc Fast solidification Arc discharge Physical vapour deposition Chemical vapour deposition Sol-gel Inert gas condensation	Ability to produce nanomaterials with uniform size, shape and distribution	Complex processes, low yield and costly types of machinery are required. This process is suitable only for highly pure materials. Hazardous by-products released during the synthesis process	Tarafdar and Adhikari (2015) and Saha and Gupta (2017)
Top-down Approach Major techniques: Etching technology Powerful ball milling cold milling or cryo-milling Severe plastic deformation Mechanical polishing and nanoimprint lithography Sliding wear	Simple, easy to handle, versatile and capable of producing large quantities Applicable for various types of materials, scalability and low cost	Non-uniformity in the formed surface characteristics makes it unsuitable for preparing uniformly shaped materials.	De Castro and Mitchell (2002)
Green synthesis	Lower cost and less toxic in nature Eco-friendly approach		Singh et al. (2015)

Types of Nanofertilizers

Based on the formulation nanofertilizers are divided into the following three major types:

(i) *Nanoscale fertilizers*: Those are formulated from conventional fertilizers by reducing the size of particles to the nanoscale.
(ii) *Nanoscale additive fertilizer*: Traditional fertilizers that contained supplement nanomaterial.
(iii) *Nanoscale coating fertilizer*: Fertilizers in which nutrients are encapsulated by nanofilms such as priming or inserted into nanoscale pores of a host material. Different materials are used for encapsulating nutrients in films or binding in nanopores within a carrier material such as to form nanocomposite structures for regulating the release of nutrients (Borges et al., 2019; Tarafder et al., 2020).

12.6.1 Nanofertilizers of Macronutrients

Macronutrients (MNs) are mineral elements that are needed in large amounts for the better growth and development of plants and for other growth-regulated mechanisms. MNs are derived from air and water, namely, carbon, hydrogen and oxygen, and those derived from soil, namely, nitrogen, phosphorus and potassium (primary MNs) and calcium, magnesium and sulphur (secondary MNs). These elements account for more than 95% of all plant biomass. Nanoscale macronutrient fertilizers can be produced by preparing nutrient emulsions in synthetic or organic polymers, encapsulation in polymeric films and adsorption on nanovehicle assembly (Barati, 2010). The *Gliricidia sepium* encapsulated urea-modified hydroxyapatite (HA) shows the gradual and controlled release of nitrogen at different pH values for crop growth and minimizes the losses of nitrogen to the environment (Kottegoda et al., 2011). According to Baloch et al. (2015), the application of macronutrient fertilizer containing NPK @ 90-45-45 kg ha^{-1} resulted in improvement in the yield and yield components of sunflowers, that is, highest plant height (144.1 cm), seed weight (46.7 g) and seed yield (2030.7 kg ha^{-1}). According to Kardaya et al. (2012), utilizing zeolite or urea-saturated zeolite as slow-release ammonia can assist in reducing the fermentation of ammonia by ruminants, the ratio of acetate to propionate, methane emission and changes in pH and reduced urea below the permitted range. Another study revealed that foliar application of 21.8 mg/L P nanofertilizer to *Glycine max* significantly increased the growth rate by 26.5 times and ground biomass by 5.4 times, respectively (Bandala & Berli, 2019). Dimkpa and Bindraban (2018) reported that foliar application Mg @ of 0.5 mg/L promotes the photosynthesis rate, induced growth and yield components in *Vigna unguiculata*.

12.6.2 Nanofertilizers of Micronutrients

Micronutrients are mineral elements that plants require in trace amounts for proper growth and development. As cofactors or the conjugating metal central atoms of several enzymes and primary/secondary biological macromolecules, these elements play critical roles in physiological and biochemical processes. Micronutrient NFs improve seed germination and root and shoot growth (possibly due to increasing planta indole acetic acid levels) and increase micronutrient content in harvest and grains.

Zinc oxide nanoparticles significantly improved biomass, enlargement of shoot and root, root surface, synthesis of chlorophyll and protein and microbial and enzymatic (phosphatases and phytase) activities in cluster bean rhizosphere, according to Raliya and Tarafdar (2013). Another study by Mahajan et al. (2011) showed that

ZnO nanoparticles improved photosynthetic activity, altered osmoregulation and reduced MDA and Na levels in lupine plants, reducing the detrimental impacts of NaCl. The maximum growth impact of nano-ZnO was observed at a concentration of 20 ppm, and the seedling's growth was restricted above this value, which could be attributed to the hazardous level of nanoparticles. According to Tarafdar et al. (2014), the application of zinc nanofertilizer increased the grain yield of pearl millet by 37.7% at crop maturity.

Montmorillonite, the main constituent of bentonite, is the most used clay in the fabrication of nanocomposite polymers. At high temperatures, a concentrated $CuSO_4$ solution is used to replace the Mg^{2+} in the clay interlayer structure. The nanocomposite prepared by binding copper nanoparticles to the vermiculite carrier makes the hybrid very strong and durable. Similarly, organoclay nanoparticles are made from kaolinite clay, which is found in lateritic and red soil and montmorillonite, which is found in black soil (Sen et al. 2015).

12.7 Fertilizers with Non-nutrient Nanoparticulates

Non-nutrient nanofertilizers are nanoparticulate materials with no nutritional relevance or contribution to the plant, such as carbon-based NMs (e.g. C_{60} fullerenes, carbon nanotubes, graphene sheets, carbon Q-dots), transition metal/metal oxide NPs (e.g. TiO_2, CdSe, CeO_2, Li, Ag, Au) and nano-clay mineral particle products. Corral-Diaz et al. (2014) found that applying nano-CeO_2 to plants increased antioxidant levels. Tripathi et al. (2017) discovered that using water-soluble carbon nano-anions resulted in significantly higher growth and yield. Furthermore, the first-generation seeds derived from plants treated with carbon nano-anions contained higher levels of protein and micronutrients. Mahmoodzadeh et al. (2013) found that 2000 mg L^{-1} increased seed germination rate vigour when compared to a control plant in another study. After the appearance of male and female flowers, nanoscale TiO_2 significantly increases the chlorophyll content such as chlorophyll a/b, carotenoids and anthocyanins in maize (Morteza et al., 2013). El-Batal et al. (2016) discovered that Ag nanoparticles significantly improve morphological characteristics and alter plant hormonal balance by accelerating the concentration of growth-promoting substances, resulting in improvement in growth and producing a significant yield of *Phaseolus vulgaris*. Application of ZnO NPs in peanuts at 1000 mg L^{-1}enhanced physiological growth and promoted earlier flowering and increased the chlorophyll content of leaves (Prasad et al. 2012). In comparison to the application of Fe-EDTA, lower concentrations (30, 45 and 60 mg/l) of iron nanoparticles increased chlorophyll content in soybean leaves (Ghafariyan et al. 2013).

12.8 Nano-biofertilizer: A New Eco-friendly Approach to Sustainable Agriculture

Biofertilizer was primarily composed of live formulations of beneficial microorganisms which improve soil aeration and natural fertilization by increasing soil moisture retention, and soil nutrient availability to plants, which helps in keeping the soil relatively healthier through the enrichment of soil microbial biodiversity. Different types of microbes utilized in biofertilizers are Rhizobium, blue-green algae (BGA), the fungal mycorrhizae, the bacterium *Azotobacter*, *Azospirillum*, which are collectively named as plant growth-promoting rhizobacteria, and phosphate-solubilizing bacteria such as *Pseudomonas* sp. and*Bacillus* sp.. However, this exciting approach has some major drawbacks, including a short life span, poor stability and lowered performance under fluctuating environmental conditions like changing temperature, radiation and pH sensitivity. Moreover, the scarcity of suitable microbial strains, the requirement of large quantities for large-scale coverage and difficulties associated with long-term use limit its vast practical field-level adaptation. Surprisingly, nano-based biofertilizer formulations have found the potential in addressing all of these critical challenges. To combat major issues of modern agriculture such as food and nutritional security, reduction of soil fertility and sustaining environmental quality, the innovative approach of nanobiotechnology plays a vital role in the development of nano-biofertilizers. The biofertilizers (containing nutrients and plant growth promoter bacteria) are coated in nanosized polymers (nanoencapsulation) for the formulation of nano-biofertilizers (Golbashy et al., 2017; Shanware & Taiwade, 2022). Dikshit et al. (2013) reported that the nano-biofertilizers produced by encapsulating beneficial microbes including growth-promoting microorganisms, namely, *Pseudomonas fluorescens*, *Bacillus subtilis* and *Paenibacilluselgii* within silver and gold nanoparticles, are strongly effective in promoting growth in various agricultural crops. The application of nano-clay polymer composite containing *Trichoderma harzianum* as biological agent significantly promoted rainfed agriculture by enhancing water and nutrient use efficiency and control of diseases, thereby increasing the productivity of rabi crops (Mukhopadhyay & De, 2014).

12.9 Nanofertilizers as a Tool for the Smart Delivery of Nutrients

With the advent of “smart fertilizers,” in a novel strategy to increase nutrient use effectiveness and lower environmental pollution, nanoscale or nanostructured materials can be employed as fertilizer carriers or vectors for the regulated release of nutrients (Chinnamuthu & Boopathi, 2009). Nanofertilizers are thought of as a unique way to provide nutrients for plant growth in a continuous and controlled way since nanoparticles have a significant capacity to deliver nutrients to target areas in biological systems. Nanofertilizers are an excellent substitute for soluble fertilizers because they release nutrients precisely and gradually over the course of the crop's growth, improving nutrient use efficiency (NUE). Nanofertilizers are much less expensive and needed in smaller amounts compared to chemical fertilizers, which are more in demand and cost more money. There are specific methods for attaching nutrition to nanoparticles. These include (a) nutrient absorption, (b) ligand-mediated attachment, (c) entrapment in a polymeric nanoparticulate shell and (d) synthesis of nanoparticles made wholly of the nutrient. Plants can receive nutrients from nanofertilizer using the following methods:

12.9.1 In Vitro Methods

12.9.1.1 Hydroponics

Gericke (1937) was the first to use this approach for dissolved inorganic salts. As the plant's roots are immersed in a nutrient solution of liquid medium, the process is also known as "solution culture" (without soil). When adopting this technique, the major factors that need to be considered are the volume of nutrient solution, aeration and pH. Different solid-supporting materials (sand, gravel and so on) are also used in this method. However, frequent disease outbreaks and wilts of plants due to high moisture rates are major downsides of this method.

12.9.1.2 Aeroponics

Weathers and Zobel (1992) were the first to report on this method. This technique includes hanging of roots of plants in the air, and the nutrient solution is sprayed continually. The gaseous environment around the roots can be adjusted using this way. However, because rapid plant development necessitates a high level of nutrients, aeroponics is not widely used.

12.9.2 In Vivo Methods

12.9.2.1 Soil Application

Chemical and organic fertilizers are often applied to the soil as a nutrient supplement. The duration of the fertilizer in the soil, soil properties (texture, soluble salt contents and pH), plant sensitivity to salts, etc. are all aspects to consider when using this form of fertilizer application. The adsorption of mineral nutrients is known to be influenced by negative soil particles. In comparison to cation exchange capacity, the exchange capacity for anions is relatively low in most agricultural soils. Nitrate, like other anions, is mobile in the soil solution and vulnerable to leaching by water flowing through it. Many cations (phosphate, iron, copper, manganese, etc.) get strongly adsorbed by soil particles which limits their mobility and availability in soil (Taiz & Zeiger, 2010).

12.9.2.2 Spray into the Foliage

This method involves applying liquid nutrients straight to the leaves. Trace elements are frequently provided by it. During the era of rapid development, foliar spray may shorten the time between applications and increase plant uptake. Insufficient nitrogen absorption from the soil is another issue that needs to be solved. Due to metal oxides' adsorbed status on soil particles and decreased accessibility to the root system, this method may be more efficient than soil application (Taiz & Zeiger, 2010). Folia spraying of nano-fertilizers can have agronomic benefits because cotyledons and leaf epidermal cells are engaged in nutrient uptake.

12.10 Mechanisms of Nanoparticle Uptake, Translocation and Fate in Plants

A recent field of interest in research is the dispersion and absorption of nano-fertilizers in plant systems. The size, shape, functionalization, stability, distribution method, plant species, age and growing environment all have an impact on the absorption, translocation and accumulation of nanoparticles. The net uptake of nanoparticle fertilizer by plants is also affected by interactions between nanoparticles and other environmental elements. For instance, humic substances increase the bioavailability and stability of nanofertilizers (Navarro et al., 2008). However, the presence of microorganisms like bacteria and fungi can affect how well plants absorb nanofertilizer (Feng et al., 2013; Wang et al., 2011).

12.11 Uptake Mechanisms of Nanoparticles by Plants

The bioavailability and cytotoxicity of NPs are determined by the order of biotransformation that takes place in the soil. After contact with plant roots, the NPs begin to migrate to aerial parts and build up in intracellular or subcellular components. The initial phase of bioaccumulation is the plant roots' absorption of nanoparticles from the soil (Nair et al., 2010). As per several researchers, plant accumulation is assumed to start with root adsorption and then diffuse into plant tissues by a variety of alterations including crystal phase breakdown, biotransformation and bioaccumulation. Since the diameter of the NPs is a crucial component that enables nanoparticles to enter via cell wall pores, it is strongly attributable to their absorption. Their size also determines how quickly they accumulate, how harmful they are and how they enter plant cells. The morphology of NPs influences their surface area, clumping and reaction on the surface of cells or inside plant structures (Wang et al., 2013). In order to determine the precise region of interactions among plant cellular components and NPs, the area and shape of the NP surface are estimated. Since the plant cell wall has a distinct negative charge, the charge of the NPs is entirely responsible for their adhesion to the surface of the plant cell.

Plant roots can be penetrated by small NPs (diameters between 3 and 5 nm) through osmotic potential and capillary pressure or directly via root epidermis (Lin & Xing, 2008). The epidermal cells of the root cell wall are semipermeable, with small holes preventing bigger NPs from entering. Some NPs made fresh pores in the cell membrane of the epidermis, making penetration simpler (Du et al. 2011; Ali et al. 2020). After passing through cell walls, NPs have transported apoplastically across extracellular spaces, enabling the xylem to travel unidirectionally upstream. The Casparian strip barrier must first be symplastically penetrated by NPs before they may reach the core vascular cylinder. This is accomplished by endocytosis, pore creation and transport of NPs to the carrier proteins of the endodermal cell membrane. NPs are internalized in the cytoplasm and transported via plasmodesmata between cells (Tripathi et al., 2017). NPs that are unable to internalize are collected by the Casparian strip, while those that enter the xylem are carried to the shoots and subsequently returned to the roots via the phloem. Plants may absorb NPs through their cell walls, cytoplasm and nuclei. Parenchymatic intercellular gaps may allow for direct NP absorption in seeds, which is accompanied by cotyledon diffusion. The cuticles or stomata are where the NPs that the leaves deposit enter the leaves. The cuticle acts as a significant leaf barrier, preventing NP entry beyond 5 nm. Through apoplastic and symplastic channels, NPs larger than 10 nm are transported intracellularly into the vascular system of plants. It is preferable to transmit NPs of 10–50 nm via the symplastic route while the larger NPs (50–200 nm) are consequently transported through the apoplastic route (Ali et al., 2020).

12.12 Mechanism of Nanoparticle Translocation in Plants

Nanoparticles that have accumulated in the plant roots move to various tissues in the plant's aerial section, including newly created seeds (Tripathi et al., 2017). Plant and NPs properties are the main determinants of nanoparticle translocation in plants. For instance, earlier research has demonstrated that *Oryza sativa* shoots can accumulate gold (Au) NPs. *Raphanus raphanistrum* and *Cucurbita pepo*'s shoots, in contrast, do not allow them to accumulate (Zhu et al., 2012). Additionally, the positively charged Au NPs are most readily absorbed by plant roots. Furthermore, compounds involving transmembrane proteins or root exudates can transfer nanoparticles to plants (Kurepa et al., 2010). Nanoparticles were discovered in other studies to penetrate the leaf through the pores or the bottom of the hyphae (Eichert et al., 2008;

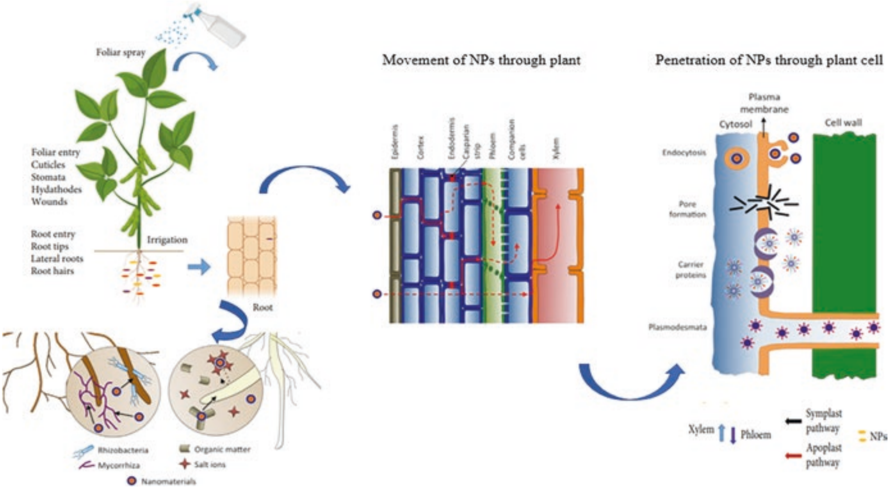

Fig. 12.1 Plant system's uptake and translocation of nanoparticles through different pathways in different plant parts described schematically

Uzu et al., 2010). Kurepa et al. (2010) observed TiO_2-alizarinred S complex absorption and translocation in *Arabidopsis thaliana* seedlings. They found that the pectin hydrogel complex which is formed by the mucilage released by roots may be the mechanism for the penetration of the nanoparticle-dye combination (Fig. 12.1).

12.13 Impacts of Application of Nanofertilizers on Ecosystems

12.13.1 Positive Impacts of Nanofertilizers

The soil serves as a reservoir for all NFs, which can enter the soil either directly or indirectly through run-offs or sewage sludge additives. Because of their engagement in crucial metabolic pathways such as nitrogen biogeochemical cycling, soil microbes are known to play a critical role in sustaining the fertility of the soil and are used as markers of soil health. Changes in the soil microbial profile can be crucial for the soil's long-term viability. The influence of NFs on soil microbial diversification and function is critical for understanding how NFs affect ecosystem functioning. The interaction of nanoparticles with environmental elements such as plants, microbes and soil has been thoroughly investigated (Dinesh et al., 2012; Pawlett et al., 2013; Tilston et al., 2013; Dimkpa, 2014; Burke et al., 2015; Simonin & Richaume, 2015; Xu et al., 2015). The fate, transport, bioavailability and consequent toxicity of nanoparticles in the soil environment are substantially influenced by the soil's physicochemical parameters (Benoit et al., 2013; Dwivedi et al., 2016). A variety of interactions exist between NPs and the soil matrix. Some scientists are researching the impact of nano-fertilizers on soil microflora viability and susceptibility. According to the study by Rajput et al. (2020), nano-fertilization boosted soil nutrients, improved soil ecosystems and increased soil microbial abundance; apart from that, the population of soil microorganisms treated with nano-fertilizer was much larger than those treated with chemical fertilizer. During the gradual release, humic acid is produced in high quantities by a nano-fertilizer. VandeVoort and Arai (2019) state that humic acid plays a vital role in maintaining soil fertility by providing carbon and nitrogen supplies for soil microbes. Furthermore, humic acid can improve soil temperature, moisture and gas permeability directly or indirectly, regulate soil pH, boost soil microbial growth and reproduction and increase the number and variety of soil microbial life. Another study found that using a slow-release nano-fertilizer boosted soil enzyme activity and microbial population in green pepper plants. Nano-fertilizer treatment boosted soil dehydrogenase and catalase activities by 37.4% and 21.3%, respectively, and increased soil bacteria, actinomycetes and fungi by 50%, 72% and 208%, respectively, as compared to the blank (Nibin et al., 2019). The antibacterial action of silver NPs on the beneficial bacterium *Pseudomonas chlororaphis* is mitigated by soil components (i.e. humic acid and soil pore water) acting as buffers (Calder et al., 2012). In *Phoenix dactylifera* leaf litter-amended soil, the application of ZnO NPs had a substantial effect on the reduction in carbon mineralization (CO_2 emissions and dissolved organic carbon), as well as nitrogen mineralization (Rashid et al., 2017). In the case of *Ipomoea aquatica*, the use of potassium- and phosphorus-incorporated NFs using zeolite as a carrier material can boost the soil's accessible phosphorus and potassium content when compared to conventional fertilizers (Rajonee et al., 2017).

12.13.2 Possible Negative Impact of Nanofertilizers

12.13.2.1 Negative Impacts on Plants

Although NFs have shown promising results in terms of plant growth, their use at higher concentrations has been shown to have deleterious effects on plant growth by oxidative stress induction as well as cytotoxic and genotoxic effects on plants and favourable soil microbes, resulting in crop growth impairment and low nutritional value (Peralta-Videa et al., 2014). Oxidative stress is induced by the adsorption and assimilation of NPs (smaller than the size of cell wall pores) by cells through interactions with plant roots. On the other hand, NPs bigger than the pores clog the pores and restrict water and nutrient absorption. Moreover, the toxicity of NPs may cause slower development and oxidative stress in plants, which results in DNA damage and alterations in genetic makeup, as well as decreased leaf colour and transpiration rate (Morales-Diaz et al., 2017).

12.13.2.2 Negative Impacts on Soil Ecosystems

Nanotechnology is a rapidly evolving technology that would have a substantial impact on food security, bioavailability, delivery system, packaging materials and emerging disease diagnosis materials in the food supply chain, helping to achieve the United Nations Millennium Development Goals. Nanotechnologies, like other new and rising technologies, provide potential benefits for farmers, consumers and the food sector. However, the safety consequences of using nanotechnologies in agriculture are little understood. The physicochemical features of these NPs, which can vary chemically and physically, determine their environmental fate. Aggregation, binding to cell surfaces (plant and microbial root), dissolution and modifications in surface chemistry are only some of the alterations that NPs can go through. For example, metal-oxide NPs may dissolve, releasing harmful metals into the environment. Furthermore, NPs may undergo homoaggregation or heteroaggregation after being released into the environment, depending on whether they interact with other NPs or mineral and organic molecules (Batley et al., 2013).

Several parameters, including pH, salinity, concentration and the presence of organic or inorganic substances, can impact a sequence of chemical reactions and nanomaterial transformations in the environment. However, synthetic nanomaterials differ from natural nanomaterials in some ways. Nanomaterials have been proven in several trials to have negative impacts on aquatic bodies and terrestrial animals, including behavioural, developmental and reproductive alterations. The bioavailability (the actual concentration of NPs to which organisms are exposed) determines their impact on soil ecosystems (Karimi & Fard, 2017). The type of soil and its features (moisture content, nature and extent of organic materials, concentration of electrolytes, acidity/alkalinity levels, aeration level and mineral composition) are likely to impact the stability and transformation of NPs. The alteration of the NFs is also influenced by biomolecules found in natural settings. Proteins, which are fundamental elements of any biological substrate, may interact with NPs and create a protein corona on their surface, resulting in surface modifications in the NPs (Hu et al., 2016). Despite the presence of proteins, electrolytes have an impact on NP dissolution and aggregation. The connection of NPs with the solid phase is also known to be influenced by the pH and humic content of the soil. Humic compounds in the soil can both stabilize and cause aggregation, depending on their content and other biotic and abiotic factors (Kralchevsky et al., 2016). Another stage of transformation is the degradation of NMs which can be caused by soil organisms or peroxidase enzymes, resulting in an increase or decrease in the by-products' toxicity. The transition of NPs causes significant changes in the properties of NMs, which must be evaluated prior to their use.

Rajput et al. (2020) conducted a study to investigate the potential hazards posed by nanoparticle toxicity to plant and microbial diversity. It was discovered that exposing the soil to nanoparticles reduced soil microbial biomass and enzyme activities, affecting microbial community composition, including yeasts, bacteria and fungi, as well as biological diversity. Similarly, plants that have been exposed to nanoparticles have developed a variety of deformities as a result of their actions.

Torsvik and Ovreas (2002) state that one of the most immediate concerns in the field of soil sustainability is the preservation of soil microbial biomass and diversity. The impact of NPs on soil is determined by their concentration, soil type and soil enzyme activity. Jiling et al. (2016) discovered that a high concentration of Fe_3O_4 nanoparticles reduced the number of microorganisms in the soil. Titanium dioxide nanoparticles reduced the number of functioning soil bacteria and enzymatic activity and had a negative impact on microbial activity, diversity and abundance (Buzea et al., 2007; Solanki et al., 2008). Another negative consequence of NPs is their impact on the rate of soil self-cleaning and nutrient balance, which is the foundation for the management of plant nutrition and soil quality improvement processes (Suresh et al., 2013).

12.13.2.3 Negative Impacts on Human Health

NPs may have a tremendous negative impact on human health considering their small size which makes them penetrate deeper into cells, tissues and organelles. These may result in chronic toxicity, DNA damage, tissue and brain damage and absorption of engineered NPs in the skin, digestive or respiratory tracts (Xia et al., 2009; Som et al., 2011). According to Lucarelli et al. (2004), silica NPs enhanced proinflammatory activity in human bone marrow monocytes. Therefore, the determination of the safety of NPs on human health needs to be critically evaluated to avoid any health hazards.

12.14 Bio-safety and Ethical Issues Related to Nanofertilizers

Nanomaterials are designed to supply plant nutrients in a regulated and slow manner. However, the risk associated with nanomaterial applications of nanomaterials has yet to be well-researched and characterized. There is a need to study the impact of nanofertilizer products on plants' physiological, biochemical, nutritional and morphological changes, as well as the concentration of nanoparticles in living systems. One of the primary concerns that the world must address before implementing nanotechnology is the hidden risks of nanoparticles, such as their hazardous effects on environmental and human health, which outweigh their potential advantages. Phytotoxicity of nanoparticles and probable residual remaining in food are two key concerns about their use in agriculture. NF levels exceeding 100 ppm were found to have detrimental effects on plants and soil ecology. As a result, choosing the doses to maximize the benefits while minimizing the hazards is critical in NF use. Boxall et al. (2007) employed algorithms to forecast the concentration of NPs in soil (i.e. direct application) and found that NP concentration is related to application rate, bulk density and depth of soil

A new field called "nanotoxicology" has emerged from the study of nanoparticles' toxicological potential as well as their proper design and application (Oberdorster et al., 2005). It is challenging to compare the safety/toxicity assessments from various research organizations because nanotechnology is so diverse and still expanding, as well as the lack of standardized methodologies and standards (Dhawan et al., 2009). Before analysing toxicological data, it is necessary to calculate the estimated concentration of nanoparticles that would be exposed to live species in the ecosystem due to the usage of nanofertilizers. The physical and chemical characteristics of nanoparticles, exposure scenario of nanoparticles from feedstuffs and toxicokinetic (uptake, delivery, biotransformation, excretion/elimination) within humans and other animal systems are some of the parameters that can be used to assess the risk associated with the use of nanofertilizers. Hence, a systematic and comprehensive quantitative investigation of the potential health risks, safe disposal of nanomaterials and environmental clearing can lead to future development and uses of nanofertilizer-based technology.

There is still no explicit legislation in Asia regarding the use of nanomaterials in the agriculture and plant production industries. However, the Japanese government's third Science and Technology Basic Plan for 2006–2010 identified nanotechnology as one of the primary study fields (Government of Japan Council for Science and Technology Policy, 2006). The Japanese Ministry of Agriculture, Forestry and Fisheries financed the "Food nanotechnology project" in 2007 as a result of the plan. Other Asian countries, such as Malaysia and India, rely primarily on national legislation, such as India's "Food Safety and Standards Act 2006", Malaysia's "Nanotechnology Industry Development Act" and "the Nanotechnology Safety-Related Act".

12.15 Future Prospects

Nanofertilizers, also known as smart fertilizers or environmentally friendly fertilizers, have the ability to enhance fertilizer use efficiency while lowering nutrient loss, particularly phosphorus and nitrogen loss. It provides the targeted place with a steady and regulated release of nutrients, preventing environmental and water body contamination. To provide nutritional quality and security in sparsely resourced areas, the perspective of a nano-biotechnology-based method for user-friendly crop management will always be acknowledged. However, the benefits and drawbacks of nano-biofertilizers should be considered since there is an urgent need to improve future research in order to reduce the risks related to NPs and bio-organic use in nanotechnology:

1. Government-based and scientific safety evaluations must be taken into account while producing nano-biofertilizers and regulating short-term organic/inorganic waste.

2. The ecosystem's biotic and abiotic components should be assessed and developed considering the impact of nanoparticles and nanofertilizers. As a result, a new method for increasing agricultural output that is both ecologically friendly and sustainable should be developed.
3. Before being used in any field operations, every novel NP-based agrochemical's physiochemical property should be thoroughly examined in long-term tests since they may directly affect health and environmental risks, limiting the compound's potential to be useful.
4. Only laboratory-based experiments could not ensure full acceptance of the nanofertilizer approach in a realistic study. Therefore, in order to provide a precise representation of the environmental impact of NPs, an experimental design must be performed in a natural environment

12.16 Conclusion

Nutrient shortage in agricultural soils has resulted in major decreases in crop productivity as well as considerable economic losses in agriculture. Although chemical fertilizers can increase crop output, their widespread use is not a long-term solution. The application of nanotechnology in agriculture includes the delivery of agrochemical substances such as fertilizers that provide macro- and micronutrients to plants. Despite the various benefits of using nanoagrochemicals over traditional agrochemicals, food safety and cytotoxicity are still major concerns. Therefore, the manufacturing of more eco- and human-friendly nanoscale agrochemicals is urgently needed concerning the safety measures of nanoagrochemicals and other risk factors related to their use and other ecological impacts. However, to understand the precise depiction of environmental impacts, scientists need to use the long-term effects of nanofertilizers and conduct detailed scientific toxicity assessments. In that situation, in addition to laboratory-based studies, more field-based implementations in the natural setting may try to comprehend the possible relationship between nanofertilizer and plant adaptations.

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Chapter 13
Promising Role of Fungal Symbiosis for Eco-friendly Green Technology for Environmental Health

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13.1 Introduction

Our planet Earth would have been colorless and lifeless without green areas and in the absence of interactions. Research has revealed how simple molecules interacted with one another to create complex substances that served as the building blocks for the emergence of life on Earth. Through natural selection and environmental pressures, simple unicellular creatures developed into more sophisticated multicellular forms that we now recognize as plant and animal life. These occurrences demonstrate how biotic (between living things) and abiotic (inside living things) interactions are essential to the maintenance of life on Earth. In this vast web of relationships, fungal symbiosis stands out as particularly important because of the positive effect it has on the plant kingdom as a whole (mycorrhizal association) (Fig. 13.1).

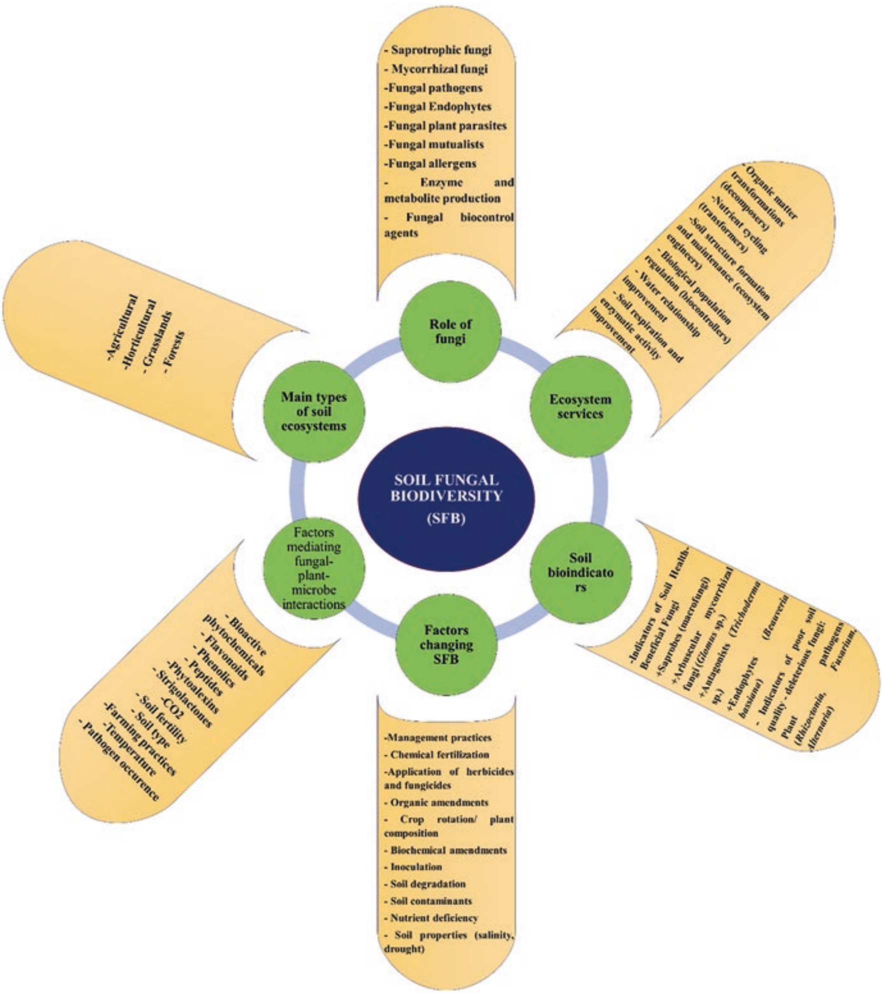

Fig. 13.1 Various aspects of soil fungal biodiversity

Mycorrhizal symbioses provide several beneficial functions to many plant species, including crop plants. Decomposition of organic matter, nutrient cycling, mineral channelization, and increased water-holding capacity are only some of the many beneficial effects of fungal symbioses on soil fertility and plant growth. Further, they inhibit the development of phytopathogens (Sommermann et al., 2018). *Trichoderma asperellum*, *T. atroviride*, *T. harzianum*, *T. virens* and *T. viride* are all commonly utilized as biocontrol agents and are commonly recognized as biostimulants for horticulture crops (López-Bucio et al., 2015). It's impossible to overstate the importance of their function in agroecosystems, food production, and cropping system efficiency (Begum et al., 2019). Soil toxicity is reduced thanks to the biosorbent effect of several species of fungi, which store toxic metals in their fruiting bodies (Frąc et al., 2018).

It is well known that plant-fungal associations improve plant resistance to abiotic stresses such as drought, salt, and temperature swings (Begum et al., 2019). Fungal symbioses, which reduce soil erosion, are an important part of sound soil management and have received a lot of attention in recent years (Chen et al., 2018). Mycorrhizal fungi also limit emissions of the greenhouse gas N_2O, which indirectly aids in ozone layer protection (Bender et al., 2014). Soil fungal biodiversity cultivation to increase soil fertility and productivity may come to be known as the "second green revolution" due to the potential impact of fungal symbioses on plant productivity and soil health (Bagyaraj & Ashwin, 2017). In this chapter, we focused on how mycorrhizal fungus can be used as an effective tool for green technology that doesn't harm the environment to increase crop yields.

13.2 Importance of Fungi in the Aspect of Plant

Recent research shows that the first plants developed from non-photosynthetic eukaryotes by ingesting cyanobacteria, which eventually gave rise to chloroplasts (Ponce-Toledo et al., 2017). A well-developed root system did not evolve until much later, so plants had to develop specialized mechanisms to extract and absorb water and nutrients from the substrate. These mechanisms include a cuticle, vascular system, and radiation defenses (Brundrett, 2002). Decedents of freshwater algae may have relied heavily on fungal symbioses when they first began to colonize land (De Vries & Archibald, 2018). Mycorrhizal fungi appear to be a monophyletic innovation that may have helped the fast colonization of landmass by vascular plants, as evidenced by recent studies, suggesting that their emergence in early land plants was a spectacular occurrence (Delaux, 2017). As the roots coevolved with the mycorrhizal symbioses in vascular plants, it is reasonable to infer that early rootless plants were involved in a wide variety of fungal connections, some of which persist today (Brundrett, 2002). The efficacy and productive services provided by mycorrhizal associations have led to their selection by the vast majority of land plants in virtually all ecological niches. Mycorrhizal symbioses, such as ectomycorrhizae, ericoid, and arbuscular mycorrhizae (Michaelson et al., 2008), are crucial for the survival and growth of many plant species, including crop plants. Mycorrhizal interactions between plant roots and fungi are some of the oldest and most common examples of plant symbiosis (Brundrett, 2002). Mycorrhiza is a symbiotic relationship between fungi and plant roots, and its name is appealing philological evincing of this relationship.

An essential substance for mycorrhizal colonizer selection in the rhizosphere is secreted by the plant's root (Mendes et al., 2013). Mycorrhizal fungi have been shown to affect practically every element of ecosystem function (Dighton, 2003), particularly soil processes including organic carbon breakdown, nitrogen, and phosphorus transformations and overall soil health maintenance (Treseder & Lennon, 2015). Mycorrhizal fungi are critical players in the nutrient cycle (Dahlberg & Bültmann, 2013), which means they are essential to the decomposition of organic materials. Furthermore, the mycorrhizal symbioses' extracellular enzymes break down organic phosphorus and nitrogen into simpler forms, hence facilitating the phosphorus and nitrogen cycle (Sinsabaugh, 1994). Mycorrhizal-interacted plants are more resistant to pathogens (Cameron et al., 2013). They aid in the plant's healthy development by increasing its resistance to pathogens, either through enhanced nutrient uptake (systemic acquired resistance (SAR)) or by priming the plant for a quicker and stronger reaction to the pathogen (induced systemic resistance (ISR)) (Conrath et al., 2006). The mycorrhizal fungi are found all over the world in nearly every major ecosystem (Rosendahl et al., 2009), and because of their central function in protecting plants from abiotic stresses (Chitarra et al., 2016), they benefit the host plant in both natural and agricultural settings (Nadeem et al., 2017). Because they help the plant absorb and translocate nutrients beyond the rhizosphere's depletion zone, mycorrhizal symbioses are key to better plant nutrition (Fig. 13.2). In addition to encouraging root system development, they interact with the host plant's phytohormone to regulate plant growth (bioregulators) and provide resistance to abiotic stress (bioprotector) (Rouphael et al., 2015; Gutjahr & Paszkowski, 2013). Mycorrhizal fungi are examined in greater depth concerning the services they provide to the host and plant, as well as the plant's productivity.

13.3 Ectomycorrhizal Fungi

The economically significant ectomycorrhizal (ECM) fungi of temperate and arboreal forests create symbiotic associations with members of the Fagaceae, Pinaceae, and Dipterocarpaceae plant families (Futai et al., 2008). Most ectomycorrhizal fungi (EcMF) are basidiomycetes, but some are ascomycetes and zygomycetes. The Hartig network mediates metabolic exchange between the fungus and the root in a symbiotic relationship with the host plant. Specifically, the mycorrhizal mantle helps in the recruitment, absorption, and transfer of soil

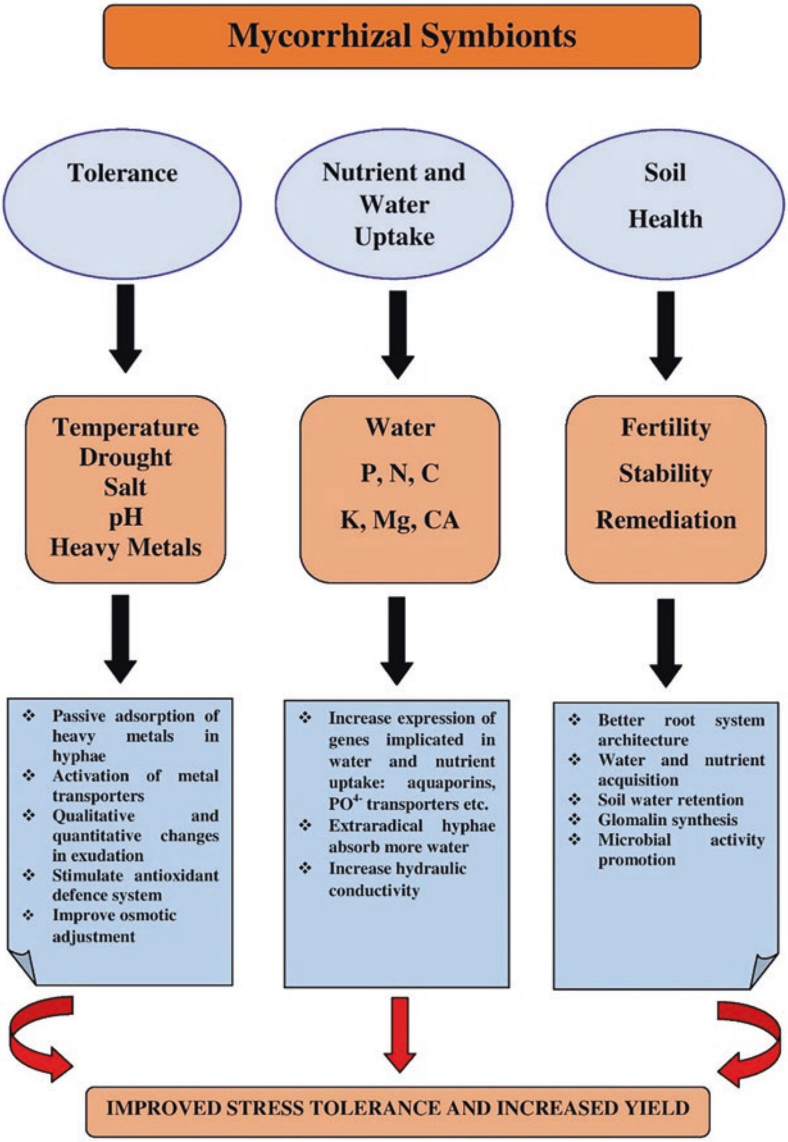

Fig. 13.2 An illustration of the mycorrhizal system's roles in controlling ecosystem processes and fostering plant development under stress conditions

nutrients and water to the roots by connecting to fungal filaments that extend into the soil (extraradical mycelium). The roots of most plant species are colonized by EcMF, which plays a crucial role in the preservation of forest ecosystems (Tedersoo et al., 2010). Scientific research has established that EcMF is crucial to the success of seedlings in a wide range of forest ecosystems (Smith & Read, 2008). Enzymes like proteases and chitinases released by EcMF break down the organic matter into simpler forms for assimilation, and this activity has been linked to EcMF's role as regulators of global carbon and nitrogen cycles in terrestrial ecosystems (Kumar & Atri, 2018; Nehls & Plassard, 2018). They increase their mycelial input and secrete organic acids and phosphatases, both of which aid in the host plant's uptake of phosphorus (Plassard & Dell, 2010). In contrast, seedlings are protected against soil pathogens thanks to EcMF colonization of the root (Laliberté et al., 2015). On top of that, EcMF can be used as a technique to lessen the impact of abiotic challenges like salt and heavy metals on the host plant (Zwiazek et al., 2019; Luo et al., 2014). For a more complete picture of how climate change is affecting the ecological impact of EcMF on the sustainability of forest ecosystems, researchers need to look closely at the link between the nutritional functioning of EcMF and their distribution pattern.

13.4 Endomycorrhizal Fungi

Zygomycota, Basidiomycota, and Ascomycota are the primary phyla that contain endomycorrhizal fungi. These endophytic fungi can infiltrate plants via spore production, where they can live in peace within plant tissues before emerging during the senescence of the host plant. Arbuscular mycorrhiza, the most prevalent type of endomycorrhiza, has been the subject of extensive study due to the role it plays in boosting plant productivity (Bonfante & Genre, 2010). The formation of an endophytic mycorrhizal symbiosis with a host plant is a multistep process (Genre et al., 2005). Endophytic mycorrhiza is formed in phases as fungal hyphae evolve and develop during root colonization (Harrison, 2012). Endophytic fungus helps plants absorb nutrients by forming a mycelial network beneath the plant's root. Common mycorrhizal networks (CMNs) are formed when a mycelial network spreads from the roots of one plant to the roots of other plants of different species (Begum et al., 2019). When these fungi and plants create a symbiotic relationship, the resulting CMN is thought to be crucial in the process of transferring nitrogen and phosphorus from the soil to the plant (Smith & Read, 2008). There is a lot of evidence to suggest that endophytic fungi can help plants resist pathogens and even prevent disease (Ownley et al., 2008). In addition, research has shown that they can enhance soil qualities, which in turn vitalize plant growth under both ideal and adverse conditions (Navarro et al., 2014). By controlling several morphophysiological changes in the host plant, fungal endophytes can reduce the effects of abiotic stresses such as drought, salt, and heavy metal toxicity (Shukla et al., 2012; Khan et al., 2011; Li et al., 2012; Hashem et al., 2015). Furthermore, endophytic fungi, in particular arbuscular mycorrhizal fungi, are recognized to operate as an environmentally benign and natural growth regulator for a wide range of terrestrial plant species. These arbuscular mycorrhizal fungi are marketed as powerful biofertilizers for long-term crop productivity, and they are employed as bio-inoculants in horticulture and agriculture (Barrow, 2012). More than 80% of terrestrial plant species have mutualistic connections with arbuscular mycorrhizal fungus (AMF). The growth of plants on land would have benefited greatly from this ancient symbiotic relationship. Genetic variation of AMF is extremely great within a species and even within a single spore, and morphometric and molecular analyses have revealed that the diversity of these fungi may be much higher (Table 13.1). Evidence suggests that inoculated soil has more extraradical hyphal mycelium than uninoculated soil (Syamsiyah & Herawati, 2018). Researchers have been drawn to these endophytic mycorrhizal symbioses because of their potential as bioregulators and bioprotectors, and various eco-friendly bioproducts for boosting plant productivity and disease resistance are currently on the market (Whipps & Lumsden, 2001).

Table 13.1 Diversity of arbuscular mycorrhizal fungi (AMF)

S. no.	AMF	Study site	Soil pH	Identification methods	References
1	*Glomus*, *Acaulospora*, *Gigaspora*, *Funneliformis*, *Septoglomus*, *Rhizophagus*, *Scutellospora*, *Cetraspora*, *Racocetra*, *Pacispora*, *Ambispora*, *Archaeospora*, *Paraglomus*, *Sacculospora*, *Viscospora*, *Dentiscutata*, and *Diversispora*	Maize (*Zea mays* L.) rhizospheres in Côte d'Ivoire	6.35	Morphometric and structural characteristics of spores	Germain et al. (2022)
2	*Glomus ambisporum, Glomus glomerulatum, Glomus microcarpum, Glomus macrocarpum, Glomus aggregatum, Funneliformis geosporum, Funneliformis constrictus, Claroideoglomus, Acaulospora laevis, Acaulospora spinosa, Acaulospora rehmii, Rhizophagus, Gigaspora, and Scutellospora*	Rhizospeheric soil of site-I (Chikadhar, Kullu District) and site-II (Jani, Kinnaur District), India	–	Taxonomic identification of AM fungal spores	Kumar and Tapwal (2022)
3	*Scutellospora rosa, Gigaspora, Acaulospora, Entrophospora, Funneliformis, Glomus and Racocetra gregaria*	Rhizosphere of *C. papaya* L. in Cameroon	5.5–6.3	Morphological and molecular analysis	Maffo et al. (2022)
4	*Gigaspora, Scutellospora*, *Glomus*, *Acaulospora*, *Archaeospora*, *Entrophospora*, and *Paraglomus*	Rhizosphere of maize in two locations in eastern Uganda	5–6.15	Morphometric and structural characteristics of spores	Fall et al. (2022)

(continued)

Table 13.1 (continued)

S. no.	AMF	Study site	Soil pH	Identification methods	References
5	*Glomus*, *Acaulospora Gigaspora*, and *Diversispora*	Rhizosphere of soybean in Benin	5.5–6.5	Morphometric and structural characteristics of spores	Houngnandan et al. (2022)
6	*Glomus*, *Gigaspora*, *Sclerocystis*, and *Scutellospora*	Rhizospheric soils of the three agroecological zones of the Central African Republic	4.32–6.08	Morphological characters of spores	DJASBE et al. (2022)
7	*Ambispora*, *Acaulospora*, *Archaeospora*, *Claroideoglomus*, *Diversispora*, *Gigaspora*, *Glomus*, *Paraglomus*, *Redeckera*, and *Scutellospora*	Rhizosphere and endosphere of a 20-year-old *Camellia oleifera*	6.13–6.51	Molecular analysis	Liu et al. (2021)
8	*Claroideoglomus claroideum*, *Claroideoglomus etunicatum*, *Funneliformis mosseae*, *Glomus coremioides*, and *Rhizoglomus intraradices*	Cover crop system at the "Chã-de-Jardim" Experimental Station, Agrarian Sciences Centre, Federal University of Paraiba, Brazil	5.01–5.89	Morphometric and structural characteristics of spores	Barbosa et al. (2021)
9	*Glomus*, *Sclerocystis*, and *Acaulospora*	Rhizosphere of medicinal plants of District Charsadda KPK	–	Morphometric and structural characteristics of spores	Yaseen et al. (2021)
10	*Ac. mellea, Ac. rugosa, Ac. spinosa, Rh. Intraradices, Rh. fasciculatum, Glomus spp.*	Legume pasture, Venezuela Centrosema Macrocarpum (field)	4.6–5.1	SSUrDNA	del Mar Alguacil et al. (2010)
11	*Ac. longula, Ac. rugosa, Ac. scrobiculata, Ac. morrowiae, Archaeospora sp., Gi. margarita, Fu. caledonius, Sclerocystis, coremioides, Rh. manihotis, Fu. Mosseae, Dentiscutata cerradensis*	Maize hybrid trials, Brazil *Zea mays* (field)	5.2	DNA (DGGE)	Oliveira et al. (2009)

13.5 Fungal Symbiosis and Soil Fertility

Soil is a finite natural resource that is vulnerable to erosion, deterioration, and pollution. Apart from this, the use of extensive agrochemicals for increasing crop productivity has put an adverse effect on soil health and fertility. Furthermore, excess application of these chemical-based fertilizers may lead to contamination and pollution of neighboring water bodies and groundwater. There is a need for eco-friendly tools for the maintenance and improvement of soil fertility. The plant–microbe interaction found in the rhizosphere is considered a major determining factor of soil fertility (Hayat et al., 2010). The fungal component regulates various crucial soil functions, such as decomposition of organic matter, cycling of nutrients, mineral channelization, soil compaction and aggregation, enhancement of water-holding capacity, plant growth regulation, and keeping a check on phytopathogens (Neemisha, 2020). The mycorrhizal fungi facilitate the carbon cycle (Fernandez et al., 2016) through the reallocation of carbon either via priming the organic matter mineralization pathway (Lindahl & Tunlid, 2015; Fernandez et al., 2016) or by fixing carbon in recalcitrant organic compounds (Sousa et al., 2012). During development, fungal symbionts form an extensive network from the mycorrhizal roots leading to the formation of a mesh or complex with the surrounding soil (Wilson et al., 2009). This mycelial network of the mycorrhizal fungi is a major contributor to total soil microbial biomass (Leake et al., 2004). The network formed by the mycorrhizal symbionts plays a significant role in binding action on the soil particle and enhances the structure of the soil. Furthermore, a hydrophobic proteinaceous substance is a glomalin (Rillig et al., 2002) which is secreted by the symbionts to facilitate soil stability and improve the water-holding capacity of the soil (Bedini et al., 2009). The mycorrhizal fungi are known to enhance the phosphorus uptake in plants growing in phosphorus-deficient soil (Parewa et al., 2010). Studies have shown that mycorrhizal association can improve nitrate ion assimilation under abiotic stress such as drought (Azcón et al., 1996). Mycorrhizal symbionts have been shown to boost the supply and uptake of micronutrients such as nitrogen, phosphorus, zinc, magnesium, manganese, and calcium to the root of the host plant and thus aid in improving soil quality and fertility (Smith et al., 1994). It is a well-established fact that the addition of biopolymers such as chitin can improve the suppressive nature of the soil as it can destroy pathogenic fungal cell walls. The soil fungal and microbial diversity regulates the suppressiveness of the soil (Cretoiu et al., 2013) and can be used as an eco-friendly technique for the eradication of soil pathogens. Moreover, studies have shown that mycorrhizal endophytic fungi can improve the tolerance of host plants to biotic and abiotic stress via ethylene or salicylic acid pathways (Lahlali et al., 2014). The evaluation and determination of the soil fungal diversity can be used as an important indicator not only for the biodiversity indexes but also to analyze the significance of the fungal diversity in soil quality and soil health.

13.6 Fungal Symbiosis in Plant Production

Soil health has declined as a result of farmers' reliance on chemical fertilizers to boost crop yields, and the impact of abiotic stress has been amplified (Begum et al., 2019). It is the symbiotic fungi, and especially the arbuscular fungi, that save the day by functioning as bio-fertilizers without harming the soil ecology. Several studies have demonstrated the beneficial effects of fungal symbiosis on plant tolerance to abiotic conditions like drought, salinity, herbivory, temperature, heavy metal toxicity, and illnesses caused by pathogenic fungi (Abdel-Salam et al., 2018). They mediate a chain of complicated communication events between the plant and the fungus, resulting in increased photosynthetic rate and other gas exchange-related features, allowing the plant to grow vigorously under adverse conditions (Birhane et al., 2012).

Plants, including crops, rely heavily on fungal symbioses for defense and resource acquisition. Mycorrhizal interactions between plant roots and fungi are among the oldest and most pervasive of all plant symbioses (Brundrett, 2004). Mycorrhiza is a fungal association with a root, and its etymology from the Greek words for both is a charming philological hint at the importance of this relationship in nature. To keep soil healthy, fungi play an important role in the decomposition of organic matter, the transformation of nitrogen, and the provision of phosphorus (Dighton, 2003; Treseder & Lennon, 2015).

Mycorrhizal fungi enhance mineral nutrition, water absorption, growth, and disease resistance in their host plants, and the plant in turn is essential for the mycorrhizal fungus to thrive and reproduce (Facelli et al., 2009). In order to maintain a stable carbon and nutrient cycle, soil fungus secrete extracellular enzymes that aid in the degradation of organic and soil components (Žifčáková et al., 2016). Wood-wide web refers to the large hyphal network created in the soil by the symbiotic fungi that connect the whole plant groups in that habitat, facilitating the efficient linear transfer of nutrients (Helgason et al., 1998). Considering the beneficial effects that fungal symbioses have on plant productivity, we can postulate that they can serve as a useful tool for environmentally responsible and long-term crop plant productivity management. It has the potential to boost agricultural output to satisfy the rising demand of the population without negatively impacting the agri-ecosystem, that is, soil health, and can act as a foundation for the development of environmentally friendly technology.

13.7 Role of Different Types of Fungi in Plant Productivity

13.7.1 Saprophytic Fungi

As the major decomposer of cellulose, lignin, and other complex organic macromolecules, saprophytic fungi are often credited with starting the breakdown process (Maltz et al., 2017; Berg & McClaugherty, 2020). Saprophytic fungi produce powerful lignocellulolytic enzymes that help break down complex organic chemicals and plant litter into simple inorganic molecules like sugars, amino acids, NH4$^+$, PO_4^{-3}, H_2O, and CO_2 that plants may easily ingest (Baldrian & Valášková, 2008). Saprophytic fungi's intricate web of hyphae makes mineralization and carbon cycling in the soil much easier (Francioli et al., 2021). Saprophytic fungi's hyphal chord can translocate carbon, phosphorus, and nitrogen, according to several studies, which aids in soil nutrient distribution (Crowther et al., 2012). Saprophytic fungi's mycelial system boosts nutrient uptake via tiny hyphae, protecting plants from the damaging effects of herbivory and grazing (Bengtsson et al., 1993). It is safe to say that the presence of saprophytic fungi improves plant productivity since they decompose organic matter, releasing nutrients into the soil.

13.7.2 Pathogenic Fungi

Pathogenic fungi are microorganisms that can cause illness in humans. They are significant actors in shaping the structure, composition, succession, and landscape patterns of a particular ecosystem. The vast majority of plant-pathogenic fungi reduce crop yields. Nonetheless, some pathogenic fungi are both friends and foes to plants. Pathogens in the soil are thought to have a significant role in the negative microbial feedback that affects species richness over broad environmental gradients. A negative effect on one species can have a positive effect on another, either by lowering competition or increasing nutrient cycling in a complex niche. Many organisms known for their harmful behavior also possess beneficial mutualistic and symbiotic traits (such as mycorrhizal fungi and endophytic antagonists found in grasses) (Winder & Shamoun, 2006). For example, the wood-rotting fungus *Phlebiopsis gigantea* has been used as a biological control tool to check the spread of *Heterobasidion annosum*, which is a causative agent of annosus root and butt rot (Roy et al., 2003). The chemical herbicides and weedicides used in agri-system also eliminate the useful microbes with the target leading to the deterioration of soil health. Studies have revealed the potency of the fungal pathogen as mycoherbicides, which can be used to keep a check on the growth of the weed population (Wall et al., 1992), which can reduce the nutrient competition, thus improving the plant productivity of that particular area. The comprehensive research studies of the niche of pathogenic fungi could reveal a more beneficial role of these pathogens.

13.8 Fungal Symbiosis and Soil Management

Soil management is a crucial step for the enhancement of the productivity of plant species especially crop plants and also for the maintenance of soil health (Bhardwaj et al., 2014). Plant experience both abiotic and biotic stresses such as drought, salinity, heavy metal toxicity, and extreme temperatures hampering the plant's growth and productivity (López-Ráez, 2016). Dependency on chemical-based fertilizers as a soil management strategy has aggravated the negative effect of these abiotic stresses on plant productivity and soil health (Begum et al., 2019). Of the known fungal symbiotes, the arbuscular mycorrhizal fungi (AMF) play a pivotal role as a bioengineer to maintain the soil structure and enhance the plant's plasticity to the changing unfavorable external condition apart from enriching the soil with essential nutrients (López-Ráez, 2016). AMF can regulate certain traits of the host such as growth, flowering, and root structure, thus helping the plant to enhance its plasticity to overcome abiotic and biotic stresses (Pozo & Azcón-Aguilar, 2007) (Fig. 13.3). During drought, AMF ameliorates a plant's sturdiness possibly by increasing surface area for water absorption through the extension of its hyphal network (Smith, S.E) or by improving the apoplastic flow of water (Bárzana et al., 2012). By promoting ion homeostasis, increasing phosphorous intake, and increasing the activity of antioxidant enzymes, Evelin et al. (2009) hypothesized that AMF reduces the stress caused by salinity. Root colonization by AMF under heavy metal stress causes the

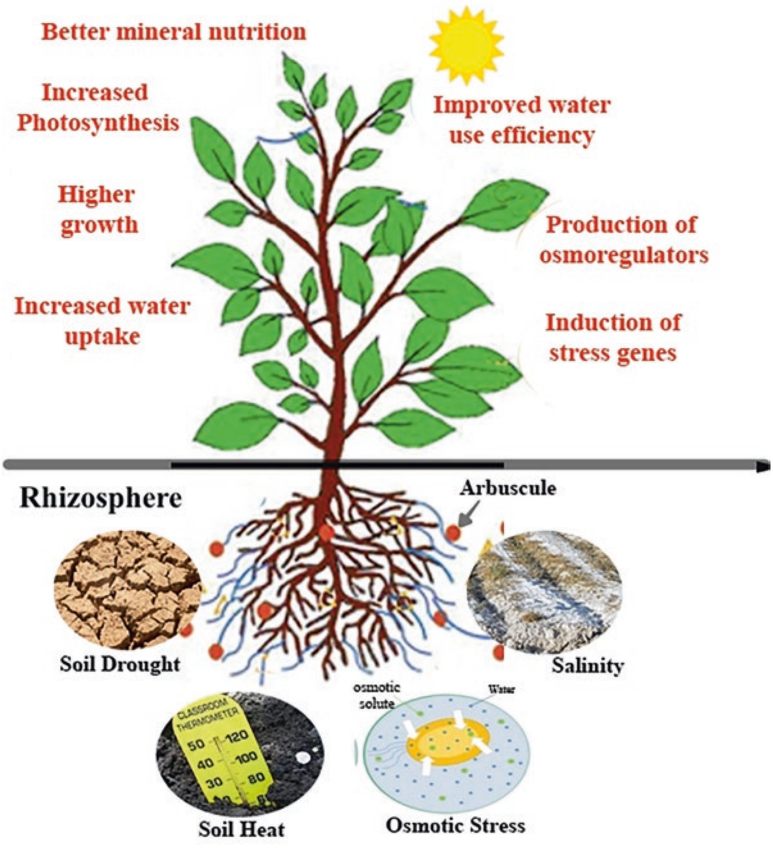

Fig. 13.3 Protective effect of arbuscular mycorrhizal fungi (AMF) against various stresses

expression of genes that control the production of proteins (metallothionein), hence increasing the host plant's tolerance to the stress (Rivera-Becerril et al., 2005).

Among the many abiotic stresses that can stunt a plant's development, high temperatures rank among the most significant. By increasing osmolyte accumulation, boosting photosynthetic activity, and shielding the plant from oxidative damage, AMF has been demonstrated to increase heat and cold tolerance in plants (Hajiboland et al., 2019; Zhu et al., 2011). Below, we will talk about the impact of abiotic stress on fungal symbioses, especially the AMF.

13.9 Soil Erosion

To put it another way, poor agricultural methods reduce the agglomeration and stability of soil particles, which leads to greater dispersal of soil particles and, ultimately, a worsening of the soil structure (Cardoso & Kuyper, 2006). Members of the soil biota have been proven to reduce soil erosion through the creation and stabilization of soil aggregates (Rillig & Mummey, 2006). Through its dense hyphal network of highly branching mycelium, which cross-links the soil particles, AMF enhances soil structure in a three-dimensional matrix (Rillig et al., 2002). The AMF hyphae produce glomalin glycoprotein, which aids in soil structure maintenance in addition to building the mesh to cross-link the soil particles (Singh et al., 2013). Soil globulin promotes carbon storage, which in turn influences soil compaction and stability (Cardoso & Kuyper, 2006). Soil aggregation is mostly dependent on globin-related soil proteins (GRSPs) (Wilson et al., 2009). Soil structure is maintained and erosion is avoided in large part because of the AMF's efforts. If you live in a dry, sandy area, you know how essential AMF's fruitfulness can be for your plants. The soil in these areas is typically poor in fertility and is easily eroded by wind and rain. Erosion can be reduced and soil fertility can be increased by planting mycorrhizal plants in certain areas.

13.10 Salinity

The salinity of soils has emerged as a major abiotic challenge in many regions of the world (Pitman & Läuchli, 2002). The salinity stress has been exacerbated by the use of chemical fertilizers and inappropriate irrigation techniques (Daei et al., 2009), which poses a severe danger to global food security. Reduced absorption due to salinity challenges suppresses plant yield (Hasanuzzaman et al., 2013), and the stress also encourages the overproduction of reactive oxygen species (Ahanger & Agarwal, 2017). The detrimental effect of salt on plant growth can be lessened by using AMF, as has been established in numerous studies (Latef & Chaoxing, 2011). This increased reliance on AMF symbiosis by plants demonstrates the crucial role it plays in mitigating the negative effects of salinity on plant growth (Tian et al., 2004). Asghari et al. (2005) found that AMF increases plant tolerance to salt stress by restoring ionic equilibrium and protecting soil enzymes (Giri & Mukerji, 2004). The photosynthetic rate, stomatal conductance, and leaf water retention of plants growing under salinity stress have recently been reported to be positively affected by AMF (Ait-El-Mokhtar et al., 2019). Chlorophyll concentration in the leaf is negatively impacted by high sodium because magnesium absorption is blocked. Sodium has a negative influence on photosynthetic rate, while AMF has been proven to boost magnesium absorption, which is necessary for chlorophyll production (Miransari et al., 2009). Plants are protected against water loss and turgor collapse thanks to the mycorrhizal symbiosis hyphal network (Abdel Latef & Miransari, 2014). Arbuscular mycorrhizal plants store organic solutes such as proline, glycine, betaine, and soluble sugars, which aid in cellular osmotic balance regulation, reactive oxygen species detoxification, and enzyme stability (Sanchez et al., 2008). Under conditions of salt stress, plants that have been inoculated with AMF have been found to have higher levels of important growth regulators such as cytokinin (Hameed et al., 2014). Plants infected with AMF have been shown in studies to produce more of the compound strigolactone, which protects them from the damaging effects of salt stress. The aforementioned function of fungal symbioses, especially AMF, demonstrates the potential of these symbioses to boost plant yield by reducing the negative impact of salinity stress.

13.11 Drought

Drought is a primary abiotic stress that has negative effects on plant development and productivity, and it is on the rise as a result of increasingly erratic precipitation (Posta & Duc, 2020). It harms crop yields and hence threatens world's food security (Zhang et al., 2018). Constraints on enzyme activity, ion absorption, and nutrient assimilation are all ways in which drought or water scarcity stress can stunt a plant's development (Ahanger et al., 2017). Stomatal closure, caused by water constraint, decreases CO_2 inflow and, in turn, affects photosynthetic activity and carbon partitioning in plants (Osakabe et al., 2014). In addition to these effects, osmotic stress brought on by dryness can produce turgor loss, which in turn suppresses plant growth and development (Kleinwächter & Selmar, 2014). Multiple investigations have also shown that plants under drought stress experience an increase in the generation of reactive oxygen species, which causes membrane damage and cell death (Gill & Tuteja, 2010). There is solid evidence from studies that the AMF can reduce plant stress caused by drought (Moradtalab et al., 2019). Water absorption is enhanced by the extensive network of AMF hyphae, which either promote apoplasticity or provide access to tiny soil pores (Augé, 2001). Osmotic adjustment and other crucial physiochemical processes are regulated by the AMF's connection with the plant (Dar et al., 2018). The stress phytohormone abscisic acid (ABA) regulates important physiological processes like transpiration rate and aquaporin expression. The stomata's closing due to ABA reactions controls water loss by limiting evaporation. By modulating ABA metabolism, AMF symbioses regulate stomatal conductance (Ouledali et al., 2019). Through its mediation of changes in other phytohormones, such as strigolactones and jasmonic acid, AMF also helps plants deal with water stress by increasing their hydraulic conductivity (Fernández-Lizarazo & Moreno-Fonseca, 2016). Superoxide dismutase, catalase, and peroxidase are antioxidant enzymes, and they are found in higher concentrations in AMF-colonized plants, where they help neutralize ROS (Ruiz-Lozano, 2003). The environmental friendliness and economic viability of sustainable agriculture methods and the management of natural resources are gaining widespread attention. To reduce the negative impact of drought stress on the plant and increase their output, AMF can be utilized as a useful tool in the development of cost-effective and environmentally friendly methods.

13.12 Temperature Extremities Stress

Human interference with the environment, such as tree cutting, burning fossil fuels, and industrial emissions of greenhouse gases, has harmed the planet's temperature. These factors have contributed to the erratic occurrence of each season and the resulting shifts in the comfortable temperature range. Negative stresses on plant growth result from temperature fluctuations outside of the ideal range (Zhu et al., 2011). Plants that are subjected to either low- or high-temperature stress have disruptions in a wide variety of crucial physiological and biochemical systems (Zhu et al., 2017). Researchers have found that plants inoculated with AMF are more resistant to high temperatures (Caradonia et al., 2019). AMF protects the plant from an unfavorable environment by boosting the water and nutrient absorption rate, photosynthetic efficiency, and osmolyte accumulation and protecting the plant from oxidative damage (Zhu et al., 2017). Heat stress reduces plant productivity by slowing development, causing leaf wilting and abscission, and senescence, changing the color of fruits, increasing oxidative stress and cell death, and decreasing yield (Wahid et al., 2007). Research shows that plants with AMF inoculation thrive under heat stress, even more so than plants without AMF inoculation (Gavito et al., 2005). When a plant is subjected to high temperatures, auxin-like morphogenetic factors (AMFs) promote root growth, allowing for more water and nutrient uptake and protecting the photosynthetic machinery from stress (Mathur & Jajoo, 2020). Damage to plant membranes caused by freezing-related dehydration is one of the most significant ways in which cold stress stunts plant development (Yadav, 2010). It was reported that low temperature leads to loss of hydraulic conductivity and disruption of stomatal regulation (Aroca et al., 2003). Plants also exhibit variations in chlorophyll content and decreased chloroplast growth (Farooq et al., 2009). Chen et al. (2013) found that inoculation with AMF improved plants' ability to withstand cold stress. Since AMF can take in and hold onto water, it helps the plant fight against dehydration (Zhu et al., 2010). Upon being inoculated with AMF, plants exhibit elevated secondary metabolite production, which boosts their immunity and protein content to better withstand the cold stress environment (Latef & Chaoxing, 2011). Chlorophyll synthesis in plants that have symbiotic relationships with an acidic bacterium (AMF) has been demonstrated to rise significantly under cold stress, aiding the plants' ability to survive and thrive despite the adverse environment (Zhu et al., 2010). The services provided by these fungal symbioses could eventually be developed into an environmentally friendly strategy to combat the effects of high temperatures on plant development and productivity.

13.13 Heavy Metal Contamination

In plant physiology, trace amounts of metals like copper, iron, manganese, zinc, nickel, cadmium, and magnesium serve as catalysts for several critical biochemical mechanisms or as cofactors of several enzymes (Nies, 1999). Soil pollution from sources such as expanding industrial zones, mine tailings, high-metal waste dumps, fertilizer applications, sewage sludge, pesticides, and coal combustion residues can lead to a buildup of heavy metals and metalloids (Wuana & Okieimen, 2011). Soil with a high concentration of heavy metals is harmful to the environment, stunts plant growth, and may even cause major health problems for humans via their consumption of contaminated food (Yousaf et al., 2016). Soil functions including filtering and buffering can be impaired by heavy metal contamination (Vamerali et al., 2010). On the other side, a plant's absorption of soil with a high concentration of heavy metals might alter the protein structure and function of enzymes (Sajedi et al., 2010). Heavy metals can also modify the structure of intrinsic proteins like H+-ATPases, which means they can affect the permeability and function of a plant's plasma membrane (Hall, 2002). Chlorosis, growth retardation, root browning, and cell cycle arrest are all toxicity signs that can be brought on by the oxidative stress induced by heavy metal toxicity (Schutzendubel & Polle, 2002). For the most part, traditional remediation methods include the costly and time-consuming process of physically relocating and storing contaminated soil. More than 80% of plants at mine sites have been proven to be colonized by AMFs, according to several studies (Wang, 2017). AMFs are essential in bioaugmentation because they help reduce plant stress caused by heavy metals in the soil. Studies have shown that AMF can aid in the phytostabilization process, which in turn reduces the negative effects of cadmium on plant growth (Janoušková et al., 2006). Colonization by AMF at the plant's roots reduces the toxicity stress caused by heavy metals by inducing the expression of genes that control the synthesis of proteins like metallothioneins (Rivera-Becerril et al., 2005). By forming vesicles or arbuscles, the fungal hyphae can immobilize the heavy metal in the cell wall, which either prevents the heavy metal from entering the plant or reduces its concentration within the plant (Hildebrandt et al., 2007). In response to heavy metal stress, arbuscular mycorrhizal association triggers the production of many antioxidant enzymes, safeguarding the plant from oxidative stress. These include glutathione S-transferase, superoxide dismutase, cytochrome P450, and thioredoxin (Hildebrandt et al., 2007). Because AMF hyphae extend well beyond the root's growth zone, it can take in extra nutrients and use them for the plant's health (a process called phytoremediation) (Leyval et al., 2002). Plants inoculated with AMF can absorb high levels of heavy metals, providing a favorable platform for removing heavy metals from soil (phytoextraction), which benefits soil health. These useful functions of AMF can be exploited as a tool to keep soil healthy, which in turn improves plant development and productivity when subjected to heavy metal stress (Christie et al., 2004).

13.14 The Potential Role of Mycorrhiza in Sustainable Agriculture

Chemical fertilizers are widely used to increase agricultural yields, but this practice has negative consequences for soil microbiology and soil health, as well as for groundwater pollution and eutrophication of aquatic bodies (Youssef & Eissa, 2014). Soil degradation brought on by agricultural malpractices has stalled crop growth (Grassini et al., 2013). An ever-increasing human population has put unprecedented strains on the world's farming and natural resource systems. Conventional agriculture techniques harm the land, water, biodiversity, and climate on a worldwide scale, while a billion people are critically malnourished today. There is an urgent need to greatly boost food production while simultaneously reducing agriculture's environmental footprint if the world is to meet its food security and growing requirements (Foley et al., 2011). Considering the aforementioned role of soil fungi in boosting crop yields in an agroecosystem, it's clear that promoting this diversity could be a useful, unique approach to meeting the increasing need for food without negatively impacting the environment (Ellouze et al., 2014). Researchers throughout the world are paying close attention to AMF symbiosis because of the many benefits it provides to plants in terms of nutrition, pathogen defense, stress tolerance, and soil structure stability (Leifheit et al., 2014; Gianinazzi et al., 2010). By allowing plants to more efficiently use vital mineral elements like nitrogen and phosphorus, biofertilization, which increases the diversity and population of microorganisms like AMF using microbial inoculants (biofertilizers), can be an environmentally preferable alternative to chemical fertilization (Alori et al., 2017). The quality of crops can be affected by AMF, and studies demonstrate that it can be used to increase agricultural yields and boost nutrient levels through biofortification (Lehmann et al., 2014). Mycorrhizal associations provide numerous advantages to plants beyond just making nutrients more accessible. By enhancing soil aggregation and, in turn, soil pore size, the vast network of mycorrhizal hyphae performs an essential function in maintaining soil structure (Mardhiah et al., 2016). Mycorrhizal symbiosis-rich soil has been found to have greater water retention capacity and higher resistance to drought stress (Ortiz et al., 2015). Mycorrhizal fungus populations have been demonstrated to decrease as a result of agricultural malpractices and disruption induced by human activities (Helgason et al., 1998). Researchers all over the world are working to improve mycorrhizal technology for the manufacturing and application of mycorrhizal inoculants as a means of combating the worldwide loss of mycorrhizal fungi (Vosátka et al., 2012).

Enhancing mycorrhizal services in the context of yield enhancement and ecological sustainability under existing socioeconomic constraints is the primary goal of mycorrhizal technology (Lamarque et al., 2014). The long-term effect of this mycorrhizal inoculant on the native mycorrhizal fungal populations is still unknown, despite benefits such as the ease of manufacture of inoculants (Schwartz et al., 2006). It is possible that fungal inoculation increases competition among the fungi within the soil which facilitates the development of species likely to offer an advantage to plants with which they associate and may lead to a desirable outcome for sustainable agricultural productivity (Field et al., 2020). In other situations, there could be the possibility of incompatibility between a crop plant and mycorrhizal genotypes, which may result in the parasitic activity of the fungi leading to increased carbon drain on the host plant, subsequently causing a reduction in yield (Klironomos, 2003). For analysis and assessment of the quality and efficacy of the inoculants and establishment of adequate standards and certification for such goods, there is a need for in-depth research and understanding of the impact of mycorrhizal inoculants on these parameters (Schwartz et al., 2006).

13.15 Need, Challenges, and Future Perspectives of Fungal Symbiosis

Researchers are particularly interested in mycorrhizal symbiosis because of the useful services it provides. Mechanisms that promote symbiosis growth, the mycorrhizal biome, the size and function of the mycorrhizal network, and a thorough understanding of the part mycorrhizal symbioses play in nutrient dynamics are all important aspects of mycorrhizal symbiosis. Significant progress has been made in recent years in identifying the key elements involved in the establishment of symbiotic interaction between plant and mycorrhizal symbioses. These elements include plant symbiotic signaling pathways, root colonization mechanisms, and host-microbe interface development (Martin et al., 2017). Our understanding of how mycorrhizal symbiosis is sustained has been expanded thanks to these investigations, and our pragmatic approach to the topic has been improved. Intraspecific variation in host plants and mycorrhizal symbioses is important for their interactions with one another and their responses to the abiotic environment, according to studies in plant physiology conducted in the light of genomes and transcriptomics (Gehring & Johnson, 2018). However, because the study of mycorrhizal symbiosis has evolved into distinct fields, only a fragmented picture of how mycorrhizae function has emerged. There is still some mystery around the genes required for initial and ongoing mycorrhizal symbiosis. A significant obstacle in understanding the relationship between plants and fungi, and the factors that contribute to the creation of the symbiotic relationship, is the investigation of symbiotic gene networks engaged in the molecular cross-talk between these two organisms. In addition, more study is needed to link molecular statistics and metabolic trails with eco-physiological and ecological processes in order to have a comprehensive understanding of mycorrhizal functioning (Van der Heijden et al., 2015). There is currently a lack of knowledge about the entire plant microbiome, which includes all fungal species that associate with plants (Hacquard & Schadt, 2015). New developments in sequencing and bioinformatics may soon make it possible to understand the intricate mycorrhizal network and the role they play within the larger context of food webs (Tedersoo et al., 2014). The physiological mechanisms driving the stability of mycorrhizal mutualism, as well as the coevolutionary processes that occur between plants and mycorrhizal symbioses, are not well known. The use of biological business models in defining the symbiotic interaction between plants and mycorrhizal fungi has potential but requires further development and testing (Van der Heijden et al., 2015). New methods and technologies for the industrial-scale production of AMF as an inoculant (Ijdo et al., 2011) have made its use in agriculture more cost-effective and reliable, leading to a greater return on investment (Ceballos et al., 2013). There is currently a need to create a biogeochemical model that can aid in forecasting the optimal time to implement mycorrhizal technology. And because of the complexity of the mechanisms involved, new mycorrhizal technologies call for extensive study. Prioritizing research into defining the characteristics controlling mycorrhizal effectiveness will help ensure that agricultural management approaches do not negate the benefits that mycorrhizae can provide. While the market for mycorrhizal products has grown in recent years, it hasn't yet reached its full potential. Mycorrhizal technology products may additionally struggle with political and legal restraints, quality assurance, and product efficacy issues, all of which are challenging but not impossible to overcome (Chen et al., 2018). There are not enough governing entities to establish quality control measures to guarantee its effective use in farming. For example, there is a lack of data on how to make an inoculant with the optimal dosage or how many propagules should be used. Mycorrhizal technology is hindered from reaching its full potential due, in large part, to the lack of public knowledge and acceptance of the technique. Although biostimulants and biofertilizers are gaining in popularity, most farmers still rely on traditional chemical fertilizer supplies. Field experiments to show the advantages of mycorrhizal technology over chemical-based fertilizers for increasing agricultural and horticultural output could be one approach to this problem (Vosátka et al., 2008). Unrealistic assumptions or expectations, on the one hand, and a sense of repeated failures in context to its effect, on the other, are preventing financial support for the development of mycorrhizal technology at this time. Uncertainty and variability are not the same, but they are often misunderstood by scientists and stakeholders alike (Lehmann & Rillig, 2014). This highlights the urgent need for communication aimed at defining the basic difference between uncertainty and variability.

The availability of sophisticated and precise technologies has made information collecting and study much simpler for the mycorrhizal scientific community. Scientists studying mycorrhiza can prove the relevance of their work to society by highlighting the function of fungi as biofertilizers, bioprotectors, and an environmentally beneficial method to boost agricultural output. We should appreciate the bountiful contributions of fungi to agricultural output and take positive steps toward improving our capacity to apply and make use of these organisms.

13.16 Conclusion and Future Insight

The world's population will reach 9 billion by 2050, according to studies (Rodriguez & Sanders, 2015). Global agricultural production will quadruple to increase food security for an expanding population. Agricultural production relies significantly on chemical fertilizers and insecticides. These methods harm the soil, ecology, and plant health. Drought, salinity, heavy metal toxicity, and excessive temperature harm agricultural yield. Heavy metal dumping from industries contaminates the soil, causing metal poisoning. Heavy metals can pollute groundwater and harm humans if they reach the food chain. Agricultural sustainability requires eco-friendly techniques. Mycorrhizal fungi help plants cope with biotic and abiotic challenges, according to studies. Mycorrhizal inoculant is a well-documented biofertilizer. Mycorrhizal fungi also reduce abiotic stressors. Mycorrhizal technology as an eco-friendly tool to boost agricultural production is in its infancy and needs additional research and field demonstration. Mycorrhiza research has traditionally been divided into separate disciplines. This isolation has left gaps in mycorrhizal knowledge (Ferlian et al., 2018). Integrate mycorrhizal research to understand mycorrhizal fungi's potential as an eco-friendly tool for agricultural output. Ecologists can help use mycorrhizal fungi for agricultural sustainability in four ways, according to Rodriguez and Sanders (2015). These aspects include determining the survival and infection capacity of inoculated mycorrhizal fungi among indigenous mycorrhizal fungi populations, the adaptability of alien mycorrhizal fungi to a foreign climate, the importance of genetic diversity among mycorrhizal fungal species and its effect on plant development, and the direct or indirect impact of inoculated mycorrhizal fungus on crop productivity. Future research should focus on identifying genes and gene products that govern mycorrhizal fungus growth and development under stress to alleviate abiotic stress on crop output. Mycorrhizal symbioses must be studied at all levels to understand their significance as biofertilizers for sustainable agriculture and to develop strategies for using mycorrhizal fungi to drive green technologies.

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Chapter 14
Nanofertilizers in Agriculture: Futuristic Approach

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Contents

14.1 Introduction

The growing world's population has forced farmers to further development of agricultural resources due to the ever-increasing demand for food with the exponential growth rate of the world's population, which exceed 9.7 billion by 2050 (United

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Nations, 2017). This futuristic food demand can only be reached by increasing resource utilization through the improvement of fertilizer use efficiency. However, the lower use efficiency of conventional fertilizers, in combination with global issues like the shortage of irrigation water and the limited land availability for cultivation, is the major challenge facing agriculture (Shah & Wu, 2019). Injudicious use of common fertilizers and their superfluous has led to environmental pollution owing to loss of nutrients through surface runoff that contaminates to natural water bodies and through leaching that causes groundwater pollution. These nontarget losses of nutrients from commercial fertilizer have been reported to be 35–40% for nitrogen, 18–20% for phosphorus, 50–55% for potassium, and 2–5% for micronutrient (Adhikari & Ramana, 2019). Additionally, the higher cost of conventional fertilizers raises as an issue for all gross root-level farmers due to their high quantities. In this context, nanotechnology is an alternative solution to overcome these problems and also is a promising smart technology to increase crop production and mitigate environmental pollution (Kim et al., 2018).

Nanofertilizers (NFs) deal with a unique prospect to develop into plant nutrients which have higher absorption rate, better control release factor, and capability of minimum nutrient losses in the ecosystem (Solanki et al., 2015). Specifically, nanofertilizers are nanoparticles having dimension of 1–100 nm in size in at least one dimension. It can serve nutrients directly or as carriers of conventional fertilizers for nutrient utilization efficiency (Ditta & Arshad, 2016). Further, nanoparticles exhibit distinct physicochemical properties of extreme ionizing power, greater reactivity, eminent absorbability, enhanced chemical stability, better pH tolerance, smaller in size, higher area-to-volume ratio, and extended thermal stability which can be considered as smart nutrient transport system (Rameshaiah et al., 2015). Nanofertilizers provide improved yield and aid in reducing the soil pollution because of overapplication of fertilizers (Naderi & Danesh-Sharaki, 2013). Nanofertilizer applications in agriculture offer a great opportunity to achieve sustainability headed for global food production.

14.2 Forms/Types of Nanofertilizers

Nanofertilizers (NFs) are defined as ingredients on the nanometer scale, usually in the form of nanoparticles which are supplied to crops in a precise mode. Modern nano-sized formulation of fertilizers increases the solubility of insoluble nutrients and their bioavailability to reduce nutrient losses (Prasad et al., 2017). The release of nutrients that are encapsulated into a particular nanocarrier is mostly stimulated by three factors, namely, biological, chemical, and physical. The biological factors are overall soil microorganisms including different bacteria and fungi that biodegrade the coating material based on their category of biodegradability, which allows the nutrient release and its fixation into the soil (Upadhyay et al., 2022a). The chemically triggered factors are pH variation, solubilization, soil type, and ion exchange

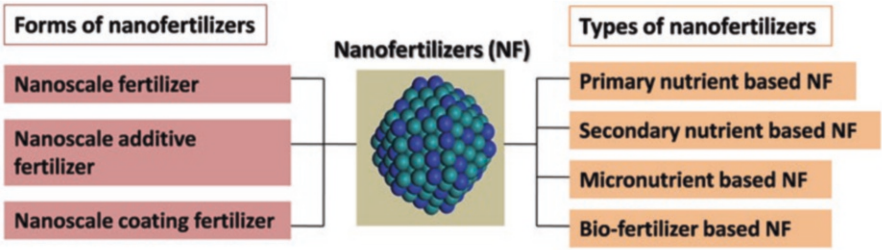

Fig. 14.1 Different forms and type of nanofertilizers used in agriculture

(Ramzan et al., 2020; Ribeiro & Carmo, 2019). The physical aspects are moisture, heat, magnetic field, ultrasound, and diffusion (Mikkelsen, 2018; Ribeiro & Carmo, 2019). Through years of research based on the type of formulation, it is possible to categorize nanofertilizers in three different forms (Mikkelsen, 2018): (i) Nanoscale fertilizer, which relates to the conventional fertilizer but in reduced size typically in the nanoparticle form; (ii) nanoscale additive fertilizer, which is a traditional fertilizer with a supplemental nanomaterial; and (iii) nanoscale coating fertilizer, which defines encapsulated nutrients by nanofilms or introduced into the nanoscale pores of a host material. Based on the type of nutrients content, nanofertilizers can be commonly categorized into three groups: (i) macronutrient-based, (ii) micronutrient-based, and (iii) biofertilizer-based nanofertilizers (Fig. 14.1).

14.2.1 Macronutrient-Based Nanofertilizers

Macronutrient-based NF is defined as the fertilizer product in which nano-sized macronutrients are coated or encapsulated. Macronutrient nanofertilizers contain one or more nutrients (e.g., N, P, K, S, Ca, and Mg), and those are required in larger amount for enhancing crop growth and higher crop yields (Chhipa, 2017). Globally, nanofertilizers are reported to have 18–29% higher nutrient use efficiency than those of conventional fertilizers (Kah et al., 2018). Thus, the highly effective and eco-friendly macronutrient nanofertilizers are seriously required to substitute conventional macronutrient fertilizer and to confirm sustainable agricultural production. Globally, the beneficial use of nitrogen-NFs in crop production has been reviewed by many researchers. Different nanocarriers like clay, zeolites, or chitosan can coordinate with plant demand and release the nutrient in a gradual pattern that improves plant absorption (Aziz et al., 2016). Nano-porous zeolites have been commonly used in the development of nitrogen-NFs due to their larger surface area as well as their ability to release the N slowly (Naderi & Danesh-Sharaki, 2013). Application of nitrogen through urea-hydroxyapatite nanofertilizer at 50% of RDF increases rice yield under field conditions (Kottegoda et al., 2017). If nitrogenous NFs are applied at the rate of 60 kg ha^{-1}, that would produce 17.6%

and 28.7% higher seed and oil yield, respectively, compared to conventional nitrogenous fertilizer (Handayati & Sihombing, 2019).

Besides, phosphorus-NFs improve phosphorus utilization efficiency and reduce the different forms of P losses. During the manufacture of hydroxyapatite nanofertilizers, some polysaccharide like carboxymethyl cellulose, sodium alginate, and lignin-coated water-soluble triple superphosphate showed persistent P release (Fertahi et al., 2020). These NFs improve the vegetation of *Adansonia digitata* (Soliman et al., 2016). Also, zeolites (surface-modified) have been described to increase P use efficiency in comparison with conventional P fertilizers (Preetha & Balakrishnan, 2017). Further, phosphorus-NFs can also be biosynthesized by introducing *Aspergillus tubingensis* TFR-5 with tricalcium phosphate (Tarafdar et al., 2012). Most commonly, commercially available phosphorous fertilizers including mono-ammonium phosphate, di-ammonium phosphate, single superphosphate, and triple superphosphate can be used on a nanoscale for better absorption of phosphates in plants (Maghsoodi et al., 2020).

Potassium-NFs show better crop response over traditional K fertilizers all over the world (Hussain et al., 2022). Numerous NPK-based nanofertilizers such as urea-modified zeolites, nano-hydroxyapatite, and mesoporous silica nanoparticles are also considered as controlled release or slow release of fertilizers (Zulfiqar et al., 2019). Among secondary nutrients, calcium is one of those important nutrients that are essential for the rapid and quality growth of plants. Peanut (*Arachis hypogaea*) cultivation with 160 mg L^{-1} calcium carbonate nanoparticles increases 14% better seed growth compared to controlled farming (no Ca) within 80 days (Xiumei et al., 2005). Magnesium is another essential secondary nutrient for crops. Mg nanofertilizers are considered more efficient than a conventional source. The application of Mg-NFs in combination with iron-NFs increases 7% seed growth of black-eyed peas (*Vigna unguiculata*) (Delfani et al., 2014).

14.2.2 Micronutrient-Based Nanofertilizers

Zinc is one of the vital micronutrients for plants (Hafeez et al., 2013). Zinc nanofertilizers play a key role to overcome zinc deficiency as they are highly reactive than its bulk form. While there are several types of Zn-NFs available such as ZnS, ZnSe, or others, ZnO-NFs is one of the most effective supplements of Zn for plants which are under investigation in the last couple of years (Sturikova et al., 2018). As an essential micronutrient, copper is incorporated in several enzymes and proteins. Cu nanoparticle-mediated fertilizers confirm the quick release of nutrients and their ready availability to the plants. Foliar application of Cu-NFs at different concentrations (50, 125, 250, 500 mg L^{-1}) improves firmness of fruit and increases abscisic acid and its antioxidant content in it (López-Vargas et al., 2018). Moreover, interest is gaining for nano-techniques in Cu in agriculture since they can serve as both fertilizers and pesticides (Wang et al., 2020a, b). Among micronutrient nanofertilizers, iron oxide nanoparticles are more efficient than chemical Fe fertilizers, having multiple inadequacies. The research described that Fe_2O_3 nanofertilizer increases root and stem growth, biomass, plant height, and antioxidant enzymes in peanut (*Arachis hypogaea*) (Rui et al., 2016). Manganese NFs as a source of Mn supplement increases the photosynthesis in mung bean (*Vigna radiata*) over conventional Mn source (Pradhan et al., 2013). Mn- along with Fe-NFs stimulates the seedling growth of lettuce from 12% to 54% (Lü et al., 2016). Molybdenum is also an important micronutrient for plants required for regular metabolism. Applications of colloidal molybdenum nanoparticles as Mo-NFs along with microorganism are found to be effective to enhance yield, disease resistance, and overall performance of chickpea under unfavorable environment (Taran et al., 2014).

14.2.3 Biofertilizer-Based Nanofertilizers

Nano-biofertilizer is a technology of encapsulation of engineering nanoparticles with microorganisms that are capable to relay sustainable agriculture by supplying sufficient nutrients to plants. It has a multipurpose approach as it contains nutrients together with plant growth-promoting microorganisms such as *Rhizobium*, *Azospirillum*, *Azotobacter*, *Azolla*, *Acetobacter*, *Bacillus*, *Beijerinckia*, *Pseudomonas*, etc. that helps in atmospheric nitrogen fixation, restoration of soil nutrients, and solubilization of insoluble complex organic matter into simple compound and releases phosphorus and other nutrients (Dineshkumar et al., 2018; Itelima et al., 2018; Upadhyay et al., 2022b). Recently, nano-biofertilizer in agriculture is considered as a promising alternative to conventional fertilizer as it is fully biodegradable and minimizes nutrient losses through controlled release (Itelima et al., 2018). Nano-size, higher surface area, and increased reactivity of nanoparticle-coated biofertilizer are the most important aspects of active nutrient acquisition for crop fertilization (El-Ghamry et al., 2018). On other aspects, the shelf life of biofertilizers is a limiting factor in these formulations, and the use of nanomaterials, namely, chitosan, zeolite, and polymers, can improve it. The stability of biofertilizers can be enhanced during nanoformulations with respect to dryness, heat, and UV inactivation. Therefore, nano-biofertilizers can solve some of those limitations of biofertilizers, but this technology yet requires some further research.

14.3 Nanofertilizer Synthesis and Mechanism

14.3.1 Synthesis

Nanofertilizers (NFs) are ecofriendly and sustainable alternatives to traditional chemical fertilizers (Babu et al., 2022). NFs showed unique physicochemical properties that make them more superior than normal fertilizers, and they open up new opportunities for sustainable and promising options for better use of fertilizers

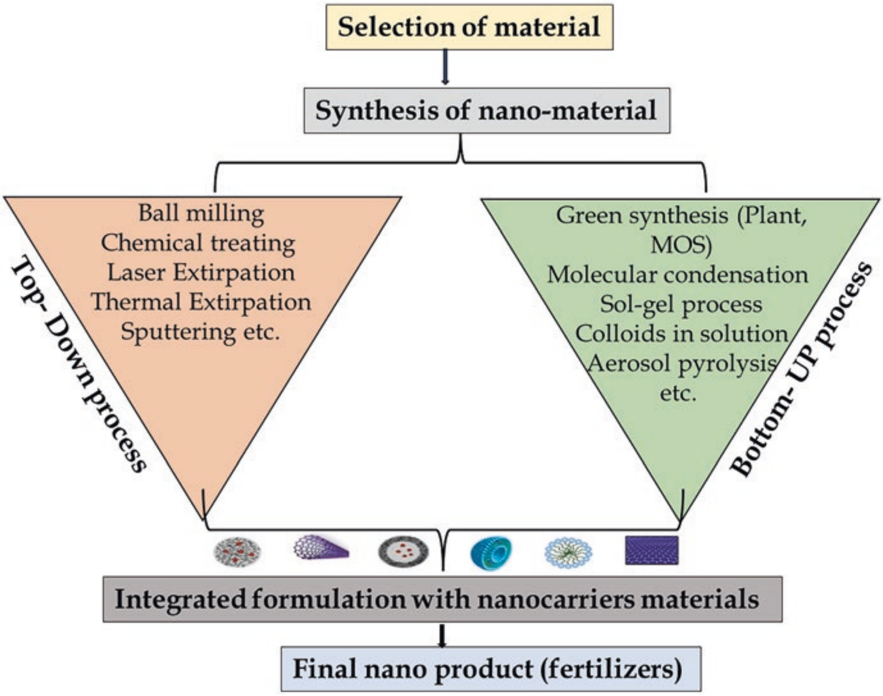

Fig. 14.2 Process of nanofertilizer synthesis

particularly in agriculture. NFs are nanoparticles (NPs) that contain nutrients and are delivered to crops in a sustained manner (Shang et al., 2019). The first step in nanofertilizer production is the selection of materials. The nanoscale fertilizer approach involves the use of nutrient-containing nanoparticles. Nanomaterials can be synthesized using both top-down and bottom-up processes (Fig. 14.2). Ball milling, chemical etching, laser extirpation, and sputtering are examples of top-down approaches, whereas in bottom-up approaches, chemical and biological methods are used (Arole & Munde, 2014). The top-down method is the reduction of bulk materials into nanoscale size. Ball milling (physical approach) has limitations in terms of nanoparticle size control and higher impurities. Physical methods are low-cost, nontoxic, and environment friendly. Biological methods are green methods of synthesizing NFs (Ganesan, 2015).

NFs can be prepared in three different ways (Fig. 14.3). Techniques like the Zetasizer, scanning electron microscopy (SEM), transmission electron microscopy (TEM), and X-ray diffraction (XRD) are used to measure and characterize the nanoparticles. Nutrient encapsulation is the most common method of producing NFs with nanomaterials. Nano-zeolite (NZ) and nano-zeolite composite materials (NZCM) were synthesized and utilized as fertilizer (Lateef et al., 2016). Nanoscale polymers were utilized in the creation of nano-biofertilizers (Golbashy et al., 2017). NFs could be designed to address a specific nutrient deficiency in plants (Babu

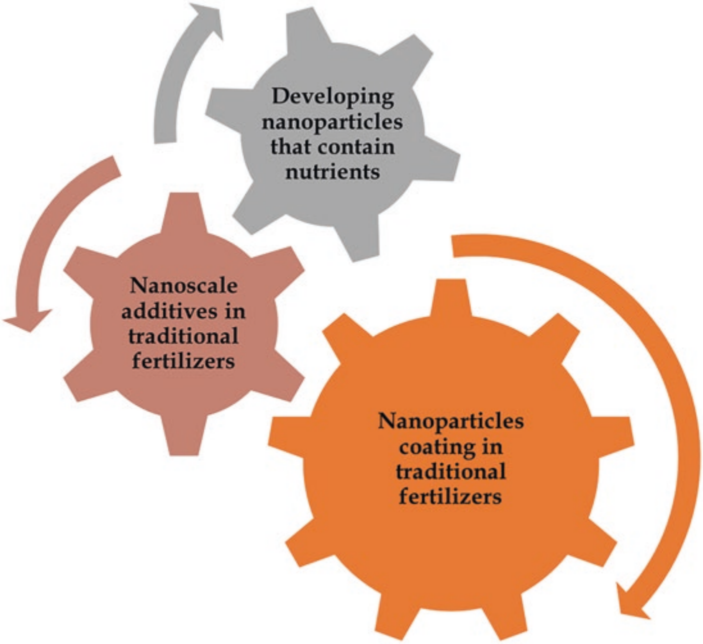

Fig. 14.3 Different ways for preparation of nanofertilizers

et al., 2022). Nanofortification of conventional fertilizers with nano-coats can be utilized to increase nutrient availability while reducing nutrient losses (Mahapatra et al., 2022). N-NFs have recently been developed by coating/encapsulating urea with hydroxy apatite. Zeolites and macro- and micronutrients are examples of materials which can be used as nanostructured nanofertilizers (NFs). Fruit peels and green plant substrates, as well as other plant materials, are used in the preparation of NFs (Sharma et al., 2014).

14.3.2 Uptake/Mechanism of Nanofertilizers

Nanofertilizers (NFs) can be applied as foliar and soil application. Nanofertilizers can be absorbed by the plant through leaves or roots. NFs that are applied to the soil enter into root and go through the xylem to aerial plant parts. NFs applied to the leaves can be absorbed by the stomata and transported to other parts of the plant via the phloem (Ebbs et al., 2016). NFs should pass through the cell wall pores in both circumstances. Only NFs with a diameter of less than 8 nanometers can pass the cell wall and reach the plasma membrane in the plant system. Nanofertilizer uptake is influenced by the application method, crop type, NF content, and soil climatic variables. Additionally, plant physiology, morphology, and anatomy all have an impact on NF penetration and translocation after foliar spray (Corredor et al., 2009). NFs are absorbed efficiently, either directly or through apoplastic and symplastic pathways, when given at the optimum (Qureshi et al., 2018). The transport of NFs from the soil to plants and from foliar spray to plant must be investigated because this information may aid in determining the efficacy of NFs. An irrigation system is the ideal technique to apply NFs if they pass through the xylem and foliar spray is recommended and appropriate if NFs are translocated via the phloem (Shukla, 2019).

Conventional fertilizers release nutrients up to 10 days, but NFs release nutrients in approximately 50 days (Seleiman et al., 2021). The N-NFs were synthesized by coating urea with hydroxyapatite nanopolymers which release N to plants gradually. Nitrogen was released 12 times slower by a nanohybrid of urea than by prilled urea (Kottegoda et al., 2017). Nanourea (liquid) is a patented IFFCO product that comprises nanoscale nitrogen. IFFCO nano-urea particles are roughly 30 nm in size. This nanourea has thousand times more surface area than granular urea. Nanourea penetrates plants more easily due to its ultrasmall size and surface characteristics. These nanoparticles release N in a regulated manner after entering plant systems. Nanourea has an 80% better uptake efficiency than traditional urea (Babu et al., 2022). Biosensor-based NFs control the pattern of nutrient delivery and bioavailability in response to crop needs (Leon-Silva et al., 2018). Figure 14.4 depicts the probable absorption mechanism parameter of nanofertilizers in plants (Ditta & Arshad, 2016).

Nanoparticles have a versatile functioning mechanism for both root and foliar entries. The size of the NPs is directly related to their absorption since it is a critical for cell wall entrances (Narayanan et al., 2020). Nanofertilizers have emerged as a viable soil management option for reducing the overuse of fertilizers. Furthermore, the slow-release method allows for different usages depending on growth stages.

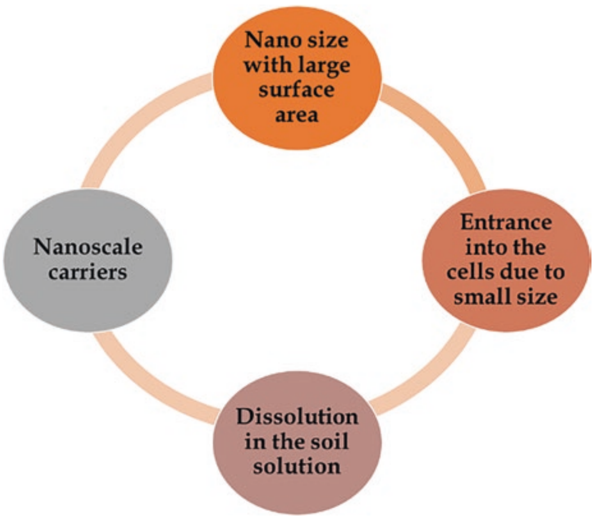

Fig. 14.4 Mechanisms of nanofertilizer uptakes in plants

The remarkable usage efficacy of nanofertilizers, as well as their high absorption rate with minimal nutrient loss, can help crops achieve maximum nutrient uptake (Mahapatra et al., 2022).

14.4 Impact of Nanofertilizers on Plant Growth

Plants need a few essential elements (macronutrients and micronutrients) for completion of their life cycle and proper growth. These are also considered as the constituents of different proteins, colors, and catalysts and engaged with cell flagging and digestion. The primary nutrients such as nitrogen, phosphorus, and potassium are utilized more frequently than any other nutrients; thus, they are required at higher rates than secondary micronutrients. Secondary nutrients are calcium, magnesium, and sulfur required by crops at smaller concentrations. The least used micronutrients are iron, manganese, zinc, copper, boron, molybdenum, and chlorine. If any one essential nutrient is missing, plants cannot germinate properly from seeds whereas the excess of nutrient on other side can harm plants. Therefore, the adequate amount of nutrient for the plants is a challenging task. A fertilizer is a vital source of soil nutrients, which plays an important role for plant growth and development. Farmers generally apply fertilizers in the soil by surface broadcasting, subsurface placement, or mixing with irrigation water. Hence, a huge quantity of fertilizers applied by these methods is lost to the atmosphere or water bodies, thereby polluting the surrounding (Tilman et al., 2002). Therefore, it is needed to develop smart fertilizers that are taken up in maximum quantity by the plants.

Nanotechnology is efficient to solve many problems, mainly nutrient losses, crop productivity, wastage of fertilizers, and many more. Nanofertilizers are made out of a few nanoparticles including metal oxides, carbon based; the nanostructure of nanofertilizers and other nanoporous materials provide a high surface area-to-volume ratio that enables to release nutrients slowly and sustainably to target sites so that soil and plants can take up nutrients easily (Rameshaiah et al., 2015). It also plays an important role in reducing nutrient leaching and volatilization losses, increasing nutrient use efficiency, providing better yield, and reducing soil pollution due to overapplication of fertilizers (Naderi & Danesh-Sharaki, 2013). This type of fertilizer plays a vital role to improve crop growth and development because of higher absorbance, high reactivity, and site specific to the cell walls. Various nanofertilizers like silica, hydroxyapatite nanoparticles, zeolite, nitrogen, copper, zinc, carbon, mesoporous silica, and polymeric nanoparticles are produced by using different carrier materials. At present, around 85% of the world's total mined phosphorus is used by farmers as fertilizers, but only 42% of the applied phosphorus the plants can uptake whereas the remaining lost to the environment making the nearby surrounding polluted. Nanoparticles have potential to influence metabolic activities of the plant in various degrees compared to traditional one and help to mobilize nutrients such as phosphorus in the rhizospheric zone (Tombuloglu et al., 2019). Zinc is one of the essential micronutrients which plays a vital part for the production of growth hormones and chloroplast (Palmer & Guerinot, 2009). Rui et al. (2016) reported that as fertilizers, iron nanoparticles play an important role to enhance photosynthesis efficiency and nutrient absorption. Copper nanoparticles are important for the formation of chlorophyll, enhancing porosity, and for few enzymatic processes (Abbasifar et al., 2020). Various organic, inorganic, and composite nanomaterials have been tested by many researchers on various plants to assess their potential impact on growth and overall development of plant. These nutrients slowly release into the active form inside plant cell which involves in the plant's cellular metabolism for their growth and development. These particles have high absorption capacity due to higher surface area-to-volume size ratio and nano-size. It helps for better uptake of nutrients from soil, leaves, or roots, resulting in the production of more photosynthates and high root and shoot biomass of crops. Several researchers reported beneficial effect of nanofertilizer on plant growth and development which are presented in Table 14.1.

Recent developments on nanoparticles in a number of crops have found enhanced germination and seedling growth, photosynthetic activity, nitrogen metabolism,

Table 14.1 Nanoparticles influence plant growth and development

Type of nanoparticle	Size (nm)	Concentration (ppm)	Mode of treatment of plant	Observation	References
Nitrogen-Urea hydroxyapatite	<200	50 kg/ha	Soil exposure	Rice slow release of nitrogen and improved rice yield	Kottegoda et al. (2017)
Phosphorus-phosphorite, Zn-induced P	<50	10–100	Soil and foliar	Cotton – Protect against oxidative stress, mobilize native P, and enhance plant growth and yield	Venkatachalam et al. (2017)
Magnesium-MgO	<10	15	Foliar	Clusterbean – Improve biomass, chlorophyll content, and phonological growth	Raliya et al. (2014)
Zinc-ZnO	20–30	10–2000	Foliar and seed	Peanut and tomato – Increase yield potential and plant growth, enhance phytochrome level and plant growth, decrease drought stress, and improve fortification	Prasad et al. (2012) and Raliya et al. (2015a, b)
Iron-iron oxide	10–100	1.5–4000	Foliar	Wheat – Increase photosynthesis rate, chlorophyll content, biomass, grain yield, and nutritional quality	Ghafari and Razmjoo (2013)
Titanium-TiO_2	5–100	200–600	Seed, soil, and foliar	Spinach – Increase plant biomass and photosynthetic activity	Linglan et al. (2008)

mRNA expression, and protein levels as well as positive changes in gene expression; these observations clearly indicate the potential use of nanoparticles for crop improvement (Kole et al., 2013). The higher level of nano-anatase enzyme led to increase in the percentage of seedling germination, various phonological characteristics, and chlorophyll content significantly (Dehkourdi & Mosavi, 2013), and good correlation was reported by Kumar et al. (2013) between the expression of key plant regulatory molecules, seed germination, growth, and the antioxidant potential of *A. thaliana* on exposure to gold nanoparticles. Titanium dioxide, a photocatalyst, has been widely studied for possible enhancement of photosynthesis (Narayanan et al., 2013). Plant height increased significantly when crops were irrigated with magnetite, hematite, or titanium dioxide nanoparticles, compared with the control treatment as reported by Ursache-Oprisan et al. (2011). Nanofertilizer applications on plants not only have positive impacts always but also some negative relations in a few studies. Magnetite and titanium dioxide showed a significant decrease in the dry weight of roots, compared with the other treatments. Titanium dioxide nanoparticles were absorbed into the stems, leaves, and fruits of tomato plants, and their exposure resulted in acute toxicity upon germination and significantly decreased root elongation at each concentration tested (Song et al., 2013). The application of nanohybrids as hydroxyapatite urea releases urea 10–12 times slower than traditional urea fertilizer as observed by Chhowalla (2017). Thus, good yields in different crops can be obtained by farmers with less nitrogen losses by using nanofertilizers and higher nutrient use efficiency (Kumar et al., 2020).

14.5 Environmental and Health Concerns of Nanofertilizers

Invention of nano-fraction of fertilizers was driven by the necessity of improved fertilizer use efficiency and better crop growth and development. Previous sections already emphasized on the synthesis and types of nanofertilizers (NFs) and their impacts on plant growth and development, particularly the beneficial aspects. Indeed, environmental and health concerns of nanofertilizers are undoubtedly most important aspects to be discussed prior to their wide acceptance and exposure.

14.5.1 Improved Nutrient Use Efficiency Vis-à-Vis Reduced Losses

Improved use efficiency could be either achieved by improving the nutrient uptake and its response or by reducing the losses. It is well known that the NFs are comparatively more soluble than traditional fertilizers; however, NFs reduced nutrient losses via absorption and fixation and improve nutrient bioavailability. Articles reported that the use of NFs could be able to improve nutrient use efficiency by 50–70%, which may be due to small size of fertilizers and high solubility which enhance nutrient uptake by plants (Seleiman et al., 2021; Kalwani et al., 2022). On the other hand, NFs reduced the applied fertilizer doses. Some research estimated that NFs are able to release nutrients in controlled modes from nanoformulations like nano-clay-polymer composites (Roy et al., 2015), nano-hydrogels (Singh et al., 2018), nano-rock phosphates, nano-glauconite, organic acid-loaded nano-rock phosphates (Roy et al., 2018a, b), or encapsulated fertilizers (Sarkar et al., 2020). Encapsulated fertilizers are controlling the nutrient release by using encapsulation as diffusion barrier (Sarkar et al., 2021). Interestingly, some of encapsulation also promote soil's native nutrient dissolution and enhance the nutrient use efficiency. Sarkar et al. (2018) reported that PVA-encapsulated controlled release rock phosphate formulations enhanced P use efficiency as well as improve P availability in the post-harvest soils, which was described as "P solubilizing microbe (PSM)-inspired chemistry" of PVA-encapsulated formulation. Some of the researchers already reported that the use of NFs may prolong the nutrient release at least by 50 days (Belal & El-Ramady, 2016; Rizwan et al., 2021) and reduced nutrient losses through leaching, because nanostructured fabrications of NFs reabsorb the excess nutrients (Shalaby et al., 2022).

14.5.2 Nanofertilizers Mediated Stress Tolerance

Because of nano-size fractions and very high specific surface area, the NFs have inordinate capability to alleviate the stresses on cultivated plants. Physiological, morphological, and biochemical indices like nutrient uptake, photosynthetic rate, their efficiency, and phytohormone regulation could enhance plant defense system. Typically, different biotic and abiotic stresses trigger protein damage, DNA damage, and oxidative stress; apart from this, plants may also show altered gene expression and altered enzyme expression (Shalaby et al., 2022). Direct or indirectly application of NFs mitigates the stress in the form of decreased generation of ROS; increases CAT, SOD, and POX enzyme activity; reduces oxidative stress (MDA and H_2O_2); activates photosynthetic genes and pigments; promotes water and nutrient uptake; regulates phytohormones; and decreases plasma membrane damage and chlorophyll degradation (Verma et al., 2022; Belal & El-Ramady, 2016; Rizwan et al., 2021; Sarraf et al., 2022; Shalaby et al., 2022; Rossi et al., 2019; Faraji and Sepehri, 2020). In specific, effects of NFs on stress resistance could be seen as individual stress (one stress is dominant), dual stress (two stress is dominant), and multiple stress (more than two stress is dominant) resistance. A list nanofertilizer-mediated stress resistance in particular plant has been presented in Table 14.2.

Table 14.2 Nanofertilizer-mediated stress tolerance to different crops

Nanofertilizers	Doses	Crop	Effects	References
ZnO	10 mg L^{-1}	Okra	Increased chlorophyll content and photosynthetic rate, improved activity of CAT and SOD enzyme, and reduced concentration of metabolites like total soluble sugars, proline, etc.	Alabdallah and Alzahrani (2020)
ZnO	15 mg L^{-1}	Arabica coffee	Improved net photosynthetic rate and vigor	Rossi et al. (2019)
ZnO	10 mg L^{-1}	Cluster bean	Improved biomass accumulation and nutrient concentration and positively affected growth physiology	Raliya and Tarafdar (2013)
ZnO	0.2 μM	Tobacco	Enriched plant development and amplified metabolite production, high enzymatic activities, and Physiology of plants	Raliya and Tarafdar (2013)
Fe_2O_3	30–90 mg L^{-1}	*Dracocephalum moldavica*	Increased the LAI, secondary metabolites, that is, phenolics, flavonoids, and pigments as anthocyanin and the boost in the catalase, glutathione reductase, ascorbate, and guaiacol peroxidase concentration	Moradbeygi et al. (2020)
$FeSO_4$	2 g L^{-1}	*Helianthus annuus* L.	High leaf surface area, dry weight of aboveground biomass, leaf area, shoot dry weight, CO_2 assimilation rate, CO_2 concentration just below stomata, Chlorophyll pigment content, Fv/Fm, and iron content with reduced Na influx	Torabian et al. (2017)
Se	10 mg L^{-1}	Sorghum	An improved antioxidant system, improved thylakoid membrane integrity, and composition	Djanaguiraman et al. (2018)
Ag	150 mg L^{-1}	Potato	Improved chlorophyll content and catalase activity	Sidkey et al. (2016)
Ag	50–75 mg L^{-1}	Wheat	Enhanced development and better heat stress tolerance	Iqbal et al. (2019)
Ag	10 mM	Wheat	Decreased antioxidative enzyme activity, increased POD activity, proline, and sugar content	Mohamed et al. (2017)

(continued)

Table 14.2 (continued)

Nanofertilizers	Doses	Crop	Effects	References
TiO_2	10–500 mg L^{-1}	Linseed	Increased carotenoids and chlorophyll content, decreased MDA activity, and levels of H_2O_2	Aghdam et al. (2016)
CuO	200 mg L^{-1}	Spinach	An enhanced physiological process like photosynthesis	Wang et al. (2020a, b)

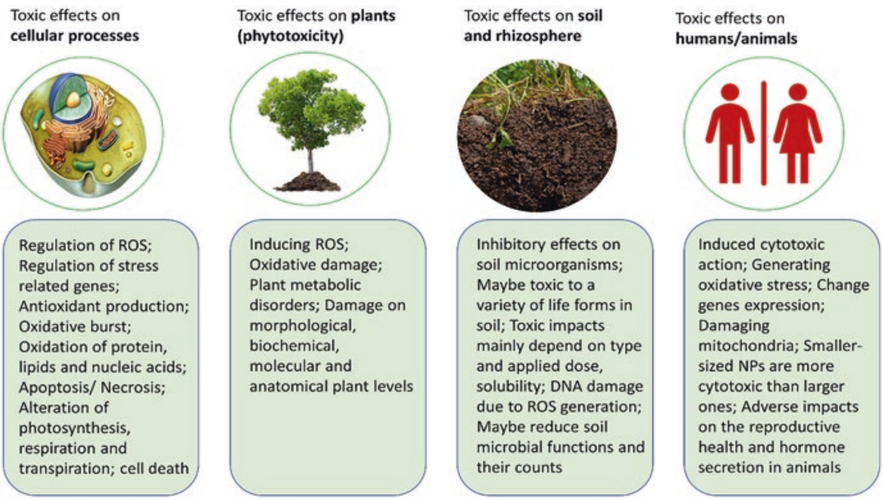

Fig. 14.5 Impacts of nanofertilizers and associates on cellular processes and plant-soil and human (animal) continuum. *ROS* reactive oxygen species. (Source: conceptualized and modified from Verma et al., 2022; Zhang et al., 2022; Kalwani et al., 2022; Bhardwaj et al., 2022; Babu et al., 2022; Zulfiqar et al., 2019; Mahapatra et al., 2022; Fatima et al., 2020)

14.5.3 Nanofertilizers and Toxicity

Though there are several beneficial effects of NFs which are already discussed, the negligence and hidden drawbacks of NF usage should be clearly discussed at the same time. Without considering the cost of production of NFs, it is a common fact that NFs are responsible for phytotoxicity of a targeted supplied material through NFs, due to its high solubility and nano-size fractions (Babu et al., 2022; Zulfiqar et al., 2019; Mahapatra et al., 2022; Fatima et al., 2020). A brief toxic effect of nanofertilizers on cellular processes, plants, rhizospheric soils, and the human/animal is pictorially presented in Fig. 14.5. Excessive use of NFs or nanoparticles could result in genotoxicity in the form of genetic mutation, chromosome fragmentation, and reactive oxygen species (ROS) formation, whereas phytotoxicity can be seen in the form of biomass reduction, chlorophyll damage, slow photosynthetic rate, and retard biomass production (Shalaby et al., 2022; Zulfiqar et al., 2019; Mahapatra et al., 2022; Fatima et al., 2020). Typically effects of NFs as phytotoxic materials could occur when it is used as the carrier of micronutrients or catalyst or as a source of heavy metals. Several researches have demonstrated that the use of metal-oxide nanoparticles (e.g., ZnO, CuO, AgO, TiO_2, CeO_2, etc.) as the nanofertilizer induced ROS generation and causes oxidative damage (Rossi et al., 2019; Wang et al., 2020a, b; Yang et al., 2015).

Several studies have reported toxicities of NFs on different crops like *Lycium barbarum* L., *Capsicum annuum*, *Pisum sativum* L., *Hordeum vulgare* L., *Oryza sativa* L., and *Solanum lycopersicum* L. (Asgari-Targhi et al., 2018; Pinto et al., 2019; Akanbi-Gada et al., 2019; Wang et al., 2020a, b; Obrador et al., 2021; Rodríguez-Seijo et al., 2022). Kalwani et al. (2022) reported the toxic effects of NFs on rhizospheric soils. Overall, the toxic effects of NFs on the human food chain and subsequent contamination of human/animal health (especially under higher doses of NFs) resulted several major issues; however, thorough study is necessary before any strict statement on the use of NFs and their effects on human health.

14.6 Advantages and Limitations of Nanofertilizers

In modern agriculture, new technologies are emerging and developed constantly, considering climate change and the environment at a focal point. Due to the overburden of natural resources, there is a need for such technology, which helps in natural resource conservation. Fertilizer is one of the important inputs in the agriculture production system. Overuse of fertilizer in the soil leads to the development of many environmental issues. Nanofertilizers have potential to achieve sustainability in global food production (Hu et al., 2017). Various types of nanoparticles are developed such as nanotubes (single or multi-walled), iron (Fe) nanoparticles (magnetized), copper (Cu), aluminum (Al), silver (Ag), gold (Au), zinc (Zn) and zinc oxide (ZnO), silica (Si), cerium oxide (Ce_2O_3), titanium dioxide (TiO_2), etc. (Raliya & Tarafdar, 2013; Raliya et al., 2015a, b; Tan et al., 2017). Researchers reported benefits as well as some limitations in the use of nanofertilizers (Fig. 14.6).

14.6.1 Advantages

1. *Slow or Control Release Nature of Nanofertilizer*

Designing of new fertilizer on the nanoscale has advantages due to the high surface-to-volume ratio, control release, and high sorption capacity (Feregrino-Pérez et al., 2018). Nanofertilizers are nutrients encapsulated or coated with nano-material (nanocarbon tubes, nanocomposites, nanofibers, nanowires etc.) for the control and slow delivery of one or more nutrients to meet the nutritional need of

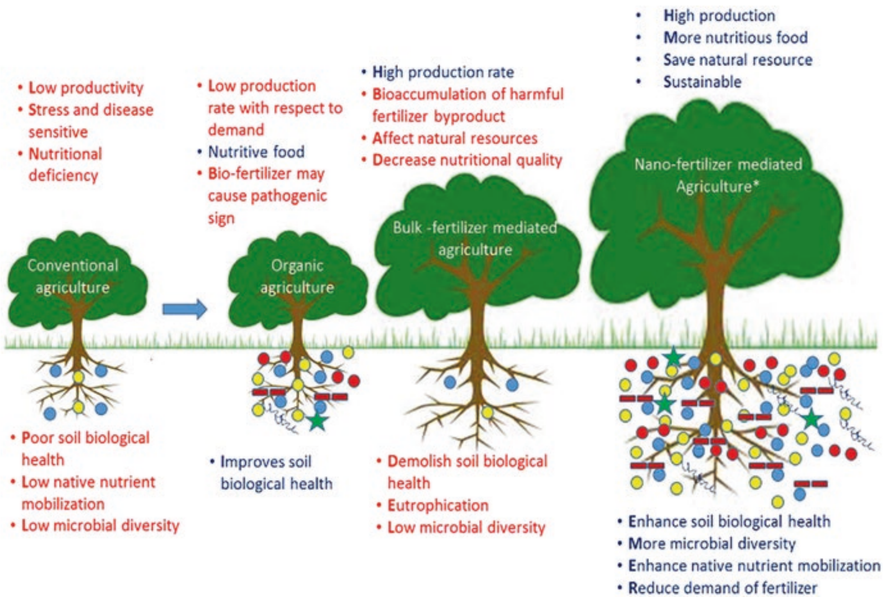

Fig. 14.6 Comparative analysis of possible pros and cons of the conventional approach with respect to nanotechnology-mediated agriculture production. The impact on the rhizosphere as well as the environment is elucidated. Red color text represents potential negative impacts of a technology. (∗) Note that the influence of the nanofertilizer further depends upon the plant growth, and the soil rhizosphere depends upon the nanoparticle type, composition, and exposure concentration. (Source: Raliya et al., 2018)

plants (Zuverza-Mena et al., 2017). Coating of such nanomaterials is linked to slow or controlled release in nature. These "smart fertilizers" are now widely viewed as a viable alternative (Rameshaiah et al., 2015); even in some cases, it is used more preferably than traditional fertilizers (Iavicoli et al., 2017). Reports suggested that slow-release nanofertilizers supply nutrients in a gradual manner up to 40–50 days during crop growth once applied while all the nutrients from conventional fertilizers release within 4–10 days (Chen & Wei, 2018).

2. *Nanofertilizer Required in Lesser Quantity Than Conventional Fertilizer*

Nanofertilizers are generally applied in smaller quantities than conventional fertilizers. Application of nanofertilizer in lower quantity reduces the chance of buildup of salts in agricultural soils over a longer period or even in short span of time (León-Silva et al., 2018).

3. *Need-Based Synthesis of Nanofertilizer*

The advantage of nanofertilizers is that they can be made to match the nutritional requirements of the crops (Kah et al., 2018). In nanofertilizer, biosensors can be linked to regulating nutrient supply based on soil nutrient status, crop development phase, or ambient environmental conditions (León-Silva et al., 2018). Reports suggested that it is very difficult to control micronutrient delivery to a particular crop in conventional nutrient management; however, nanofertilizers allow producers to give optimal levels of nutrients (Feregrino-Pérez et al., 2018).

4. *Enhancing Bioavailability of Nutrients*

Due to very high specific surface area, smaller size, and strong reactivity, nanofertilizers boost nutrient bioavailability and efficient absorption of nutrient by plants (Liu & Lal, 2015; Kyriacou & Rouphael, 2018).

5. *Beneficial Effect on Growth and Yield of Plant*

In a greenhouse experiment, Liu and Lal (2014) synthesized a novel form of hydroxyapatite ($Ca_5\ (PO_4)_3OH$) nanoparticle with a size of 16 nm and evaluated fertilizing effects of nano-hydroxyapatite on soybean (*Glycine max*) in an inert growth medium. They reported that nano-hydroxyapatite enhanced growth rate and seed production by 33% and 20%, respectively, in comparison to conventional phosphatic fertilizer ($Ca(H_2PO_4)_2$). In another study, Liu et al. (2005) found that the application of calcium carbonate ($CaCO_3$) nanoparticles (20–80 nm, 160 mg L^{-1} as Ca) as a Ca nutrient to peanut (*Arachis hypogaea*) seedlings cultivated in the sand with Hoagland solution for 80 days dramatically increased seedling development compared to control where no Ca was applied. In comparison to conventional Mg and Fe fertilizer, combined foliar application of Mg and Fe nanoparticles on black-eyed pea (*Vigna unguiculata*) enhanced the weight of 1000 seeds by 7% (Delfani et al., 2014). In a greenhouse experiment under a hydroponic setting, Ghafariyan et al. (2013) found that low concentrations of superparamagnetic Fe-nanoparticles substantially increased the photosynthetic pigments (chlorophyll content) in sub-apical leaves of soybeans, implying that soybeans might use this type of nanoparticle as a source of Fe and reduce chlorotic symptoms of Fe insufficiency. Application of ZnO nanoparticle in lower concentration (20 mg L^{-1}) enhances growth and development of mung bean and chickpea. In the case of mung bean, root length and root biomass enhance by 42% and 41%, respectively, while the corresponding value for the shoot was 98% and 76%, respectively (Mahajan et al., 2011). Further study by Zhao et al. (2013) suggested that ZnO nanoparticles improve cucumber (*Cucumis sativus*) growth when applied at the rate of 400 and 800 mg kg^{-1} to a soil mixture.

6. *Additionally, Nanofertilizers Save Shipping and Application Expenses (Fan, 2014)*

Nanofertilizers, on the other hand, enable the plant to withstand diverse biotic and abiotic challenges by delivering balanced nutrition, with demonstrable benefits. However, there are certain critical limitations to using nanofertilizers in agriculture that must be recognized.

14.6.2 Limitations of Nanofertilizers

Recent advancements in sustainable agriculture have surely seen the effective usage of several nanofertilizers for increasing crop output. However, the intentional use of this technology in agricultural activities might have a number of unforeseen and irreversible consequences (Kah, 2015). New environmental and unforeseen health safety risks might restrict the application of this technology in the production of different crops. Phytotoxicity of nanomaterials is also a concern as different plants respond to different nanomaterials differently in dose-dependent ways (Ashkavand et al., 2018). As a result, it is critical to assess both the benefits and limits of nanofertilizers before putting them on the market. Nanomaterials, in particular, are highly reactive due to their small size and increased surface area (Konate et al., 2018). These materials' reactivity and variability are also a source of worry. This raises concerns about the safety of agricultural workers who may be exposed to xenobiotics during application and manufacturers who expose during manufacturing (Nair, 2018). Apart from the different benefits of nanofertilizer, it is necessary to investigate the feasibility and applicability of these novel smart fertilizers. Concerns regarding their transportation, toxicity, and bioavailability, as well as unanticipated environmental effects from exposure to biological systems, limit their use in sustainable agriculture and horticulture (Feregrino-Pérez et al., 2018). Nanomaterial risk assessment and hazard identification, as well as nanomaterial or fertilizer life cycle evaluation and toxicological research, are essential. This is particularly relevant in the context of nanoparticle accumulation in plants and associated health risks. Phytotoxic effects of nanoparticles were also reported (Ebbs et al., 2016). However, toxic effect depends on absorption, translocation, transformation, and accumulation by plants as well as dose application technique and type of nanoparticle (Ebbs et al., 2016).

14.7 Future Prospects

In 2011, worldwide macronutrient fertilizer usage ($N + P_2O_5 + K_2O$) was 175.7 million tons (Mt), with a projected rise to 263 Mt. by 2050 (Alexandratos & Bruinsma, 2012). According to Smil (2002), N fertilizers have provided a 40% rise in per-capita food production over the last five decades, demonstrating the importance of these macronutrient fertilizers in the world's food supply. Furthermore, considerable amounts of these nutrients (N and P) are transferred into surface and groundwater bodies due to the low use efficiency (30–50%) and intensive application of these macronutrient fertilizers, altering aquatic ecosystems and posing a health risk to humans and aquatic life. As a result, developing highly efficient and environmentally friendly macronutrient (N and P) nanofertilizers to replace traditional N and P fertilizers and assure sustained food production while safeguarding the environment is an urgent and practically vital research path. Thus, macronutrient nanofertilizer development is a top goal in fertilizer research. Nanofertilizer production process and deployment are still in their early phases; there is little if any particular study or systematic studies on the impacts and benefits of applying micronutrient nanofertilizers in the field. Another issue with employing nanomaterials as fertilizers is nanotoxicity. To assuage public concerns about nanotoxicity, more study on the toxicity of a newly developed nanofertilizer should be done. Zeolites remain a potential nanomaterial for decreasing N leaching and efficiently enhancing N usage. More study is needed to evaluate the yield and environmental advantages of N-loaded zeolite fertilizers to the additional expenses associated with zeolite procurement and the N-loading procedure. Other forms of nutrient-augmented nanoparticles, such as silica NPs, iron oxide NPs, and carbon nanotubes, should also be researched and developed. Comprehensive growth-promoting mechanisms must be investigated. Any success on crop production stimulators other than nutrients would be a turning point not just for nanotechnology research but also for bringing in a new "Green Revolution" (Liu & Lal, 2015).

14.8 Conclusions

Nanofertilizers are a relatively recent topic in the literature that is constantly evolving. For synthesis of nanofertilizer, different methods, namely, top-down and bottom-up approaches, were adopted. Ball milling, chemical etching, laser extirpation, and sputtering are examples of top-down approaches whereas bottom-up approaches use chemical and biological methods. Researchers reported that nanofertilizers have a substantial influence on increasing production and resilience to abiotic stressors. As a result, the potential of nanofertilizers in food production cannot be underestimated. Furthermore, in the present climate change scenario, the potential benefits of nanofertilizers have sparked a lot of interest in increasing the productivity of agricultural crops. Although there are more benefits than drawbacks to using these nanomaterials, the study in this field is still preliminary and extremely specific. As a result, much more research is needed to establish this technology as a viable, safe, and sustainable option for agricultural use and eliminating associated hazards.

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Chapter 15
Plant Secondary Metabolites and Their Impact on Human Health

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15.1 Introduction

The plant kingdom creates a huge number of low-molecular-weight organic molecules (Matthias & Daniel, 2020). According to their function in fundamental metabolic processes, the phytochemical components of plants are typically divided into two groups called primary and secondary metabolites. Based on the ascribed roles for these substances, the scientific community has functionally categorized them

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into three: (1) primary metabolites, (2) secondary metabolites, and (3) hormones (Hussein & EL-Anssary, 2018). Primary metabolites are very important for plant growth (Fernie & Pichersky, 2015); secondary metabolites facilitate plant interactions with abiotic and biotic factors (Hartmann, 2007) and phytohormones, which control processes in the organism and the synthesis of other metabolites by interacting with receptor proteins (Davies, 2004; Upadhyay et al., 2022a, b).

Primary plant metabolites are more or less similar in all living cells because they are involved in basic life processes (Hussein & EL-Anssary, 2018). However, secondary plant metabolites are subsidiary processes of the shikimic acid pathway. The plant kingdom has more than 50,000 secondary metabolites. Secondary metabolites have been found to be multifunctional in the course of the investigation; they can serve as medicinal impact of herbals, which is focused on secondary plant metabolites, relieving different ailments in traditional medicine and folk applications. In modern medicine, they contributed lead molecules for the creation of drugs to treat a range of illnesses, from cancer to migraine. The primary categories of secondary plant metabolites are phenolics, alkaloids, saponins, terpenes, and lipids.

The physiology of plants and developmental stage of the plant's mineral nutrients affects production of secondary metabolites modulated by growth condition and environmental factors (Li et al., 2018; Clemensen et al., 2020). In many recent literature, the most popular method for assessing how nutrition affects plant secondary metabolites involves physiological changes brought on by plant growth conditions through analysis of metabolite profiles in response to supra- or sub-optimal nutrient concentrations and analysis of their impact on the development, growth, and biosynthesis of the plant secondary metabolites. For instance, stress caused by nitrogen, phosphate, potassium, and sulfur induced the biosynthesis of phenylpropanoids and phenolics in a number of plant species. There are lots of secondary metabolites that we can eat to improve our health, for example, carotenoids that are found in plants, algae, and photosynthetic microorganisms in their natural forms. In general, most of the carotenoids come from fruits and vegetables. The vibrant colors of pumpkins, sweet potatoes, cantaloupes, papayas, and tomatoes are derived from carotenoids, which are red, orange, and yellow (Khoo et al., 2011). The body obtains the majority of its carotenoids from leafy greens like spinach. Lycopene and zeaxanthin are two important carotenoids, which contain antioxidants that clean the human body of reactive oxygen and nitrogen species; zeaxanthin and lycopene consumption has implications for the prevention of cancer (Rao & Rao, 2007). Flavonoids are found naturally in plants and mainly found in tomatoes, mango, and litchi. Similar to carotenoids, flavonoids have antioxidant effects (Jideani et al., 2021). Free radicals damage the circulatory system's endothelial walls and may be a factor in atherosclerotic alterations. Flavonoids protect the circulatory system's walls and lower the risk of heart disease by scavenging free radicals (Lobo et al., 2010). Additionally, flavonoids inhibit the development of tumors, osteoporosis, and viral infections (Nijveldt et al., 2001). Glucosinolate is produced from amino acids which are present in cruciferous vegetables like broccoli, collard greens, cabbage, and mustard as the dietary source of humans. It acts in controlling the amount of replicating cells in a region of uncontrollable cell development by triggering apoptosis for the prevention of cancer. They also possess antioxidant capabilities, which help to defend the body against oxidative stress (Traka & Mitchen, 2008). Allium species, including onions and garlic, contain a category of terpenoids called saponins, and they are also rich in spinach, tea, and legumes. By attaching to and eliminating cholesterol from cell membranes, saponins help to maintain heart health. The danger of injury to the heart is increased by a stiff vascular system. By attaching to that cholesterol and removing it from artery membranes, saponins can stop that from happening (Böttger & Melzig, 2013). Hence, consuming saponins is thus one way to support and help in improving heart health (Marangos et al., 1984). However, a variety of conditions have an impact on their production, altering plant mechanisms. The adverse environmental stress and climatic factors are the primary stressors that influence plant physiology and have a stimulating effect on secondary metabolites in crops and medicinal plants (Wink, 2015; El-Hendawy et al., 2019). In several plant species, productions of secondary metabolites are very low which can be improved by altering biotic and abiotic elicitors and application of biotechnological tools.

15.2 Plant Secondary Metabolites

Plant synthesizes numerous low-molecular-weight organic compounds using simple inorganic compounds, and based on their potential functions, these compounds are basically divided into three classes, namely, primary metabolites, secondary metabolites, and hormones (Fig. 15.1). Primary metabolites include amino acids, common sugars, protein, and nucleic acids such as pyrimidines and purines, chlorophyll, etc. (David, 1995). Unlike primary metabolites, plant secondary metabolites (PSMs) are not essential for normal growth, development, and multiplication of living cells (Fraenkel, 1959). The word secondary often implies that this group of metabolites may not be very significant for plants but this however is not true considering the benefits and impacts of this group of metabolites. Most of the PSMs provide protection to plant from any possible damage or harm in the ecological environment (Stamp, 2003) andother possible interspecies protection (Samuni-Blank et al., 2012). PSMs may not be universal to all plants, but extraction of these metabolites from all known sources is said to be engaged in large number of biological activities. The PSMs have gained importance particularly in the fields of medicines, drugs, cosmetics, pharmaceuticals, and chemicals or recently in the field of nutraceutics (Tiwari & Rana, 2015). In fact about 25% of the total molecules used in pharmaceutical industries are of plant origin (Payne et al., 1991). For example, the active molecule in aspirin, that is, acetylsalicylate, is isolated in huge amount from plants like *Betula lenta* and *Spiraea ulmaria* (Payne et al., 1991). In the last few decades, these metabolites have been an important topic of research owing to their immense potential in human health care.

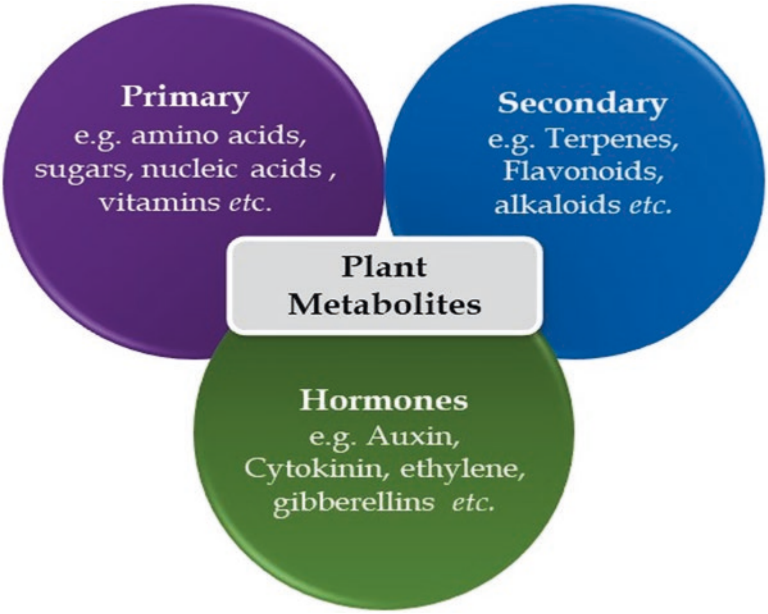

Fig. 15.1 Types of plant metabolites

15.3 Sources of Plant Secondary Metabolites

Plant secondary metabolites (PSMs) are generally considered products with high economic value and act as active ingredients in certain chemical products like medicines, flavoring agents, perfumes and fragrances, insecticides, pesticides, dyes, etc. (Thirumurugan et al., 2018). As the name suggests, these PSMs are mainly produced inside plants through various biosynthesis pathways. In common, plants are the source of 80% of secondary metabolites, and the rest is contributed by microorganisms (bacteria, fungi) and different marine species such as sponges, snails, tunicates, and corals. Table 15.1 enlists the different sources of secondary metabolites and their probable numbers (Berdy, 2005), and similarly, Table 15.2 enlists some important secondary metabolites and their source. Many more secondary products from these sources are continuously being researched upon.

15.4 Biosynthesis Pathways

Metabolites are synthesized through biochemical pathways, and their production process requires energy source which is obtained from adenosine triphosphate (ATP) (Herbert, 1989). These pathways mainly operate utilizing the energy produced during tricarboxylic acid cycle (TCA) cycle and glycolysis of carbohydrates

Table 15.1 Secondary metabolites and their abundance in nature

Sources	Identified compounds	Bioactive compounds	Antibiotic compounds
Natural products	More than a million	0.2–0.25 million	25,000–30,000
Plant kingdom	600,000–700,000	150,000–200,000	~ 25,000
Microorganisms	More than 50,000	22,000–23,000	~17,000
Algae and lichens	3000–5000	1500–2000	~1000
Higher plants	500,000–600,000	~100,000	10,000–12,000
Animal kingdom	300,000–400,000	50,000–100,000	~5000
Protozoa	Several hundreds	100–200	~50
Invertebrates	100,000	NA	~500
Marine organisms	20,000–25,000	7000–8000	3000–4000
Insects, worms, etc.	8000–10,000	800–1000	150–200
Vertebrates (mammals, fishes, amphibians, etc.)	200,000–250,000	50,000–70,000	~1000

Source: Berdy (2005)
NA – data not available

Table 15.2 Important secondary metabolites and their sources

Source	Secondary metabolites	References
Plants		
Papaver somniferum	Morphine and codeine	Siah and Doran (1991)
Sapium sebiferum	Tannin	Neera and Ishimaru (1992)
Torreya nucifera var. radicans	Diterpenoid	Orihara and Furuya (1990)
Capsicum annuum	Capsaicin	Johnson et al. (1990)
Allium sativum	Alliin	Malpathak and David (1986)
Azadirachta indica	Azadirachtin	Sujanya et al. (2008)
Cassia acutifolia	Anthraquinones	Nazif et al. (2000)
Cornus kousa	Polyphenols	Ishimaru et al. (1993)
Eriobotrya japonica	Triterpenes	Taniguchi et al. (2002)
Polygala amarella	Saponins	Desbène et al. (1999)
Camellia chinensis	Flavones	Nikolaeva et al. (2009)
Arachis hypogaea	Resveratol	Kim et al. (2008)
Gentiana macrophylla	Glucoside	Tiwari et al. (2007)
Microorganisms		
Streptomyces sp. BD21–2	Bonactin	Schumacher et al. (2003)
Streptomyces chibaensis	Resistoflavine	Gorajana et al. (2007)
Streptomyces sp. M491	Chalcomycin A and terpenes	Wu et al. (2007)
Bacillus coagulans	Coagulin	Le Marrec et al. (2000)
Bacillus amyloliquefaciens FZB42	Bacillomycin	Ramarathnam et al. (2007)
B. subtilis	Bacilysocin	Tamehiro et al. (2002)
P. stutzeri KC	Pseudomonine	Lewis et al. (2000)
Penicillium raistrickii	Oxaline	Sumarah et al. (2011)
Monascus ruber, *Aspergillus terreus*	Lovastatin	Dewick (2009)

(Kabera et al., 2014). The production of ATP takes place from the catabolism that involves oxidation of primary metabolites like amino acids, fats, and glucose. Adenosine triphosphate utilized here is reutilized in anabolic processes involving intermediate molecules of the pathways. Catabolism occurs through oxidation whereas anabolism process requires reduction, and hence, there is a need for reducing agent which is usually the NADP (nicotinamide adenine dinucleotide phosphate). The catalyst for the reaction is coenzyme, and the most dominant coenzyme A (CoA) is made up of ADP (adenosine diphosphate) and pantetheine phosphate which is chiefly responsible for donating or accepting hydrogen in anabolic and catabolic reactions, respectively (Michal & Schomburg, 2013). Biosynthesis of glycosides and polysaccharides occurs through pentose phosphate pathway whereas biosynthesis of phenols occurs through shikimic acid pathway (Kabera et al., 2014). Acetate malonate pathway leads to biosynthesis of alkaloids, and mevalonic acid pathways steer the biosynthesis of steroids and terpenes (Dewick, 2002). Fig. 15.2 briefly outlines the process of biosynthesis of PSMs through the

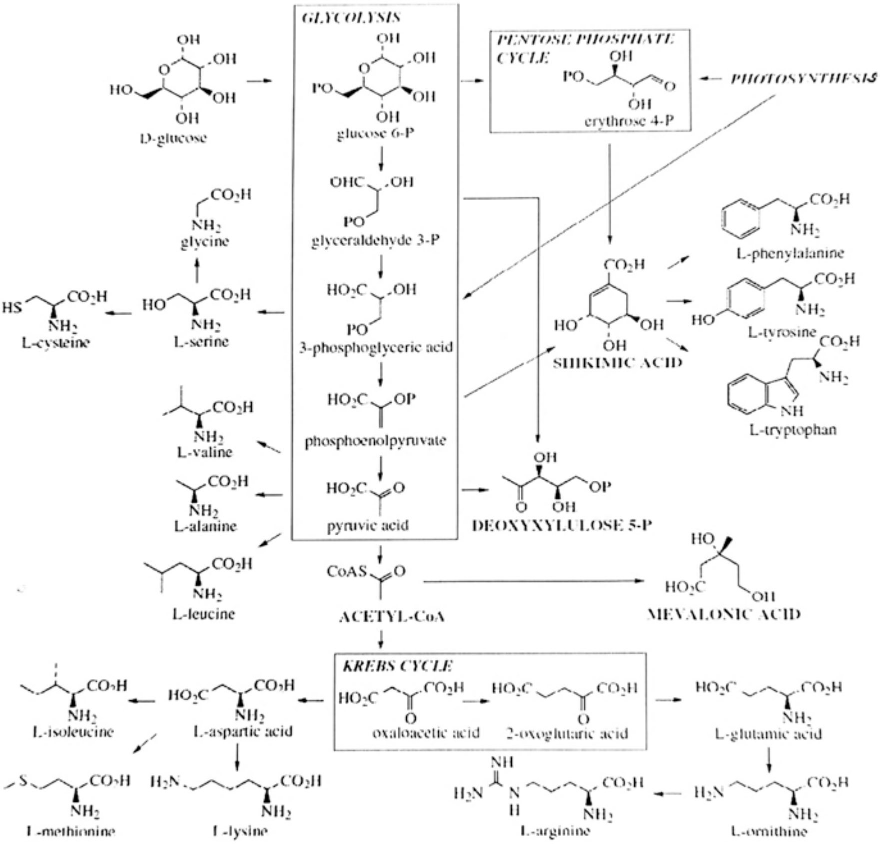

Fig. 15.2 Schematic representation of biosynthesis of plant secondary metabolites. (Adapted from Kabera et al., 2014)

process of photosynthesis, glycolysis, TCA, or Krebs cycle. Generally, the important building block involved in the biosynthesis of secondary metabolites is derived from acetyl-CoA (acetyl coenzyme A), shikimic acid, mevalonic acid, and 1-deoxylulose 5-phosphate ((Kabera et al., 2014). Commercial production of these secondary metabolites may be done through modified synthetic pathways either from primary metabolites or from substrates having primary metabolite origin. Apart from chemical synthesis, PSM production was earlier achieved through cultivation of medicinal plants; however, it is a very time-consuming method. Plants originating from particular biotopes were not easy to cultivate outside their existing local environmental conditions; also there were problems of pathogen sensitiveness. Also the amount of PSM produced in nature is very meagre and thus requires immense harvesting to obtain sufficient quantities of these molecules for preparation of botanical drugs, etc. As a result, plant cell, tissue, and organ culture approach was considered by scientists and biotechnologists as an alternative way for PSM production (Thirumurugan et al., 2018). These culture techniques can be used in a routine manner under aseptic conditions from explants such as roots, shoots, leaves, meristems, etc. for both extraction and multiplication purposes. The process of in vitro production of PSM has been used and reported from commercial medicinal plants. Zenk (1991) were able to observe that differentiated cell culture from commercial medicinal plants could produce anthraquinones @2.5 g/l of medium. This was the beginning of an era of use plant tissue cultures for the production of PSM of pharmaceutical and industrial interests (Bourgaud et al., 2001). This method showcased some real life practical advantages over conventional method which are listed as follows:

1. There was no dependency on climate and soil conditions for production of PSMs, and useful molecules can be produced under controlled conditions.
2. Since cultured cells would be prepared under aseptic conditions, it would be devoid of microbial contamination.
3. Metabolites produced under harsh climates can also be easily produced and multiplied in laboratory conditions.
4. Automatic system of regulation of cell growth would reduce the labor cost to a great extent and improve productivity.
5. Substances with organic origin could also be extracted from callus cultures.

Due to these benefits, research in the area of tissue culture technology for production of PSM has become quite popular in recent years.

15.5 Classification of Plant Secondary Metabolites (PSMs)

More than 2.14 million secondary metabolites have already been identified, and their vast diversity in structure, function, and biosynthesis serves as the basis of classification of PSMs. Basically, PSMs are classified into three broad groups, that

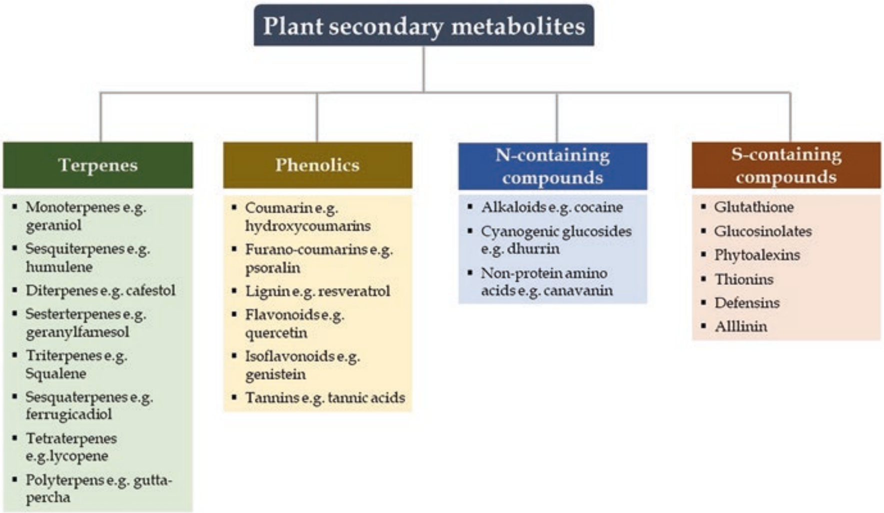

Fig. 15.3 Types of plant secondary metabolites. (Source: Twaij & Hasan, 2022)

is, terpenes, phenolics, and nitrogen- and sulfur-containing compounds. The types of secondary metabolites under each broad category have been outlined in Fig. 15.3.

15.5.1 Terpenes

This class of PSM is considered as one of the most dominating groups among all secondary metabolites. It is a group of most active compounds having more than 23,000 known structures. Structurally, terpenes are hydrocarbon-based natural product with isoprene (5-carbon units) as the basic unit. These polymers of isoprene derivatives are synthesized from acetate via the mevalonic acid pathway. Their classification is based on number of units incorporated into a particular terpene.

They have a general formula of C_5H_8 where depending upon value of n, it is classified as monoterpenoids, diterpenoids, triterpenoids, and so on. These terpenes have pharmacological importance and are used for treatment of ailments in both humans and animals. Recently, the potential of this group of metabolites to showcase antihypertensive activity has been discovered which can represent a new era of medicine (Kabera et al., 2014). Besides, they also have antibacterial and insecticidal properties which make them important for manufacturing of insecticides and pesticides for agricultural and horticultural use to counter the biotic stress (Kabera et al., 2014). The monoterpenoids include menthol, eugenol, and camphor which are reported to be possessing high antioxidant property. Certain groups of diterpenoids like resins and taxol have been identified to have anticancer properties. The triterpenoids like cardiac glycosides, ursolic acid, and steroids possess significant cytotoxic, sedative, and anti-inflammatory properties (Velu et al., 2018).

15.5.2 Phenolics

Phenolic groups of secondary metabolite are characterized by the presence of a hydroxyl functional group (phenol group) on aromatic ring. Phenolics act as a major defense system against pest, diseases, and pathogens including root-infesting nematodes. They have anti-inflammatory, anti-oxidative, anticarcinogenic, antibacterial, and anti-helminthic properties and also provide protection from oxidative stress (Park et al., 2001). These are produced by plants which are recognized to have health benefits like vegetable, fruits, tea, cocoa, etc. These groups of secondary metabolites occur in almost all plants and are subjected to a number of biological, agricultural, chemical, and medical researches (Dai & Mumper, 2010). Phenolics are classified on the basis of (i) number of hydroxylic groups; (ii) chemical composition, namely, mono-, di-, oligo-, and polyphenols; and (iii) number of aromatic rings and carbon atoms in the side chain, for example, phenolic with one, two aromatic rings, quinones, and polymers. Polyphenols are further subdivided into flavonoids and non-flavonoids like tannins. Flavonoids are found in vacuole of plant cell as water-soluble pigments which are further subdivided into anthocyanin, flavones, and flavonols. Tannins are also water-soluble compound, and they can form the complex with proteins, cellulose, starch, and different minerals. Their synthesis is mainly governed by shikimic acid pathway, also known as the phenylpropanoid pathway as it also leads to the formation of other phenolics such as isoflavones, coumarins, lignins and aromatic amino acids, etc. (Kabera et al., 2014).

15.5.3 Nitrogen- and Sulfur-Containing Compounds

This group is inclusive of phytoalexins, defensins, thionins, and alliin which have been associated directly or indirectly with the defense of plants against various microbes having pathogenic activity (Grubb & Abel, 2006). Glucosinolate (GSL) is a group of low-molecular-mass N (nitrogen) and S (sulfur) containing plant glucosides that is produced by higher plants. This GSL imparts resistance against the unfavorable effects of predators, competitors, and parasites because when it breaks down, certain volatile-defensive substances are released exhibiting toxic or repellent effects (Jamwal et al., 2018). Nitrogen-containing PSM includes alkaloids, cyanogenic glucosides, and nonproteins amino acids, and most of N-containing PSMs is biosynthesized from amino acids (Jamwal et al., 2018). Alkaloids are compounds composed of nitrogen, carbon, oxygen, and hydrogen, but in some cases, elements like phosphorus, chlorine, sulfur, and bromine may also be present in the alkaloid structures (Nicolaou et al., 2011). They are found in approximately 20% of the species of vascular plant and primarily responsible for defense against microbial infection. The primary, secondary, and tertiary amines responsible for the basic nature present in the alkaloid groups are classified on the basis of the number of nitrogen atom existing in the alkaloid group (Velu et al., 2018). The extent of basicity of alkaloids depends upon the variation in the chemical configuration of the molecular structure and the occurrence of various functional groups at different locations in the alkaloid molecule (Sarker & Nahar, 2007). Some important alkaloids are morphine (used as analgesics), berberine (used as antibiotics), vinblastine (with anticancer properties), etc. Apart from these, other important alkaloids include codeine, nicotine, coniine, cytisine, solanine, quinine, strychnine, tomatine, etc.

15.6 Functions of Secondary Metabolites in Plants

Secondary substances have signaling functions, influence the activities of other cells, regulate their metabolic activities, and coordinate the development of the whole plant. Other substances, such as flower color, help communicate with pollinators and protect plants from animal damage and infection by producing specific phytoalexins after fungal infection and fungal hyphae within plants, and inhibits body diffusion (Mansfield, 2000). Plants also use phytochemicals (such as volatile essential oils and colored flavonoids or tetraterpenes) to attract insects for pollination and other animals for seed dispersal. Compounds belonging to terpenoids, alkaloids, and flavonoids are currently used as pharmaceuticals or dietary supplements to treat or prevent various diseases (Raskin et al., 2002) and cancer (Reddy et al., 2003; Watson et al., 2001). It is estimated that 14–28% of higher plant species are used for medicinal purposes, and 74% of pharmacologically active plant constituents were discovered after ethnomedical use of plants (Ncube et al., 2008). Secondary metabolites are metabolic intermediates or products that occur as a product of differentiation in restricted taxa, are not essential for the growth and life of the producing organism, and are derived from one or more common metabolites. They are biosynthesized via more diverse metabolic pathways (Mansfield, 2000). The presence of volatile monoterpenes or essential oils in plants provides plants with an important defense strategy, especially against herbivorous pests and pathogens. Volatile terpenoids also play important roles in plant interactions and act as pollinator attractants (Tholl, 2006). They function as signaling molecules and show evolutionary relationships with their functional roles. Soluble secondary compounds such as cyanogenic glycosides, flavonoids, and alkaloids can also be toxic to animals.

15.7 Benefit of Plant Metabolites in Human Health

1. Anti-inflammatory and Antioxidant Compounds.

Diseases like diabetes, cancer, and photoaging can be attributed to inflammation as their causative agent or triggering factor. Inflammatory responses modify the transcriptome by upregulating several transcription factors and pro-inflammatory cytokines of our tissues. A solution to unresolved inflammation could be plant bioactive compounds that exhibit natural anti-inflammatory activities, often in conjugation with antioxidant properties (Teodoro, 2019).

2. Neuroprotective Compounds.

The neurological disorders such as Alzheimer's disease, multiple sclerosis, Parkinson's disease, neuropathic pain, etc. can be traced back to neuro-inflammation. Both age-related conditions and age-independent pathologies could lead to neuro-inflammation via similar cascade. About two million people worldwide die of cerebral ischemic diseases every year. Some remedies for this condition come from herbal sources (Teodoro, 2019). The effect of total flavonoids was studied from *Abelmoschus esculentus* L. against transient cerebral ischemia reperfusion injury (Lau et al., 2008). It was suggested that the protective effects were due to direct or indirect antioxidant actions via free radical scavenging or activation of Nrf2-ARE pathway, respectively. Oxidative damage plays an important role in neuronal damage, which may proceed to cause neurodegenerative diseases such as Alzheimer's disease. Pequi, a phytomedicine derived from *Caryocar brasiliense* of Caryocaraceae family, is known to be a potential neuroprotective medicine. de Oliveira et al. (2018) reported the mechanism of this neuroprotective effect to be due to anticholinesterase or antioxidant properties. Some procyanidins, extracted from lotus seedpod, exhibited anti-Aβ effects in rat models.

3. Anticancer Compounds.

Nowadays, several plant bioactive compounds are gaining importance as anticancer agents. Several studies also show that these natural components increase the efficacy of chemotherapy and sometimes even reduce the side effects of chemotherapeutic drugs (Ramakrishna et al., 2021). Four such plant-based bioactive compounds, namely, curcumin, myricetin, geraniin, and tocotrienols, are well known for their anticancer properties (Subramaniam et al., 2019). A major class of vitamin E, tocotrienol, is also known for its anticancer properties. It is present in plant products like rice bran oil, palm kernel oil, etc. (Aggarwal et al., 2010). Both in vitro cell-based studies and in vivo animal model experiments proved that tocotrienol exhibits antitumor properties and prevents proliferation of cancer cell lines such as pancreatic, liver, stomach, lung, and breast cancers (Ramakrishna et al., 2021).

4. Antiviral Effects of Plant Bioactive Compounds.

Bioactive herbal extracts have long been used to treat ailments such as viral infections. In the current situation of the COVID-19 pandemic, we can rely on some of these plant bioactive secondary metabolites as antiviral agents. Human coronaviruses, such as the COVID-19 severe acute respiratory syndrome (SARS) coronavirus (Geller et al., 2012) and the Middle East respiratory syndrome coronavirus (MERS-CoV), can cause the common cold, which primarily affects the respiratory tract, but there is no vaccine. Sometimes it turns out to be fatal. Naturally occurring terpene iodine glycosides such as saikosaponins (A, B2, C, and D) found in *Bupleurum* spp., *Heteromorpha* spp., and *Scrophularia scorodonia* are known for their antiviral activity against one of the human coronaviruses HCoV229E (Cheng et al., 2006).

15.8 Effect of External Factors on Plant Secondary Metabolites

Plant secondary metabolites (PSMs) are generally unique sources of medications, food additives, flavors, and biochemicals of industrial significance. In plants exposed to various elicitors or signal molecules, the buildup of such metabolites frequently occurs. Secondary metabolites are crucial for a plant's environmental adaptability and stress tolerance. The effects of temperature, humidity, light intensity, water availability, minerals, and CO_2 on plant growth and the synthesis of secondary metabolites are many (Akula & Gokare, 2011). Examples of environmental conditions that have a detrimental effect on plant development and agricultural productivity include drought, high salt, and cold temperatures. The various outside variables that affect plant secondary metabolites are as follows:

Influence of Salt Stress The presence of salt in the environment encourages cellular dehydration, which results in osmotic stress and water loss from the cytoplasm and a decrease in the volumes of the cytosol and vacuoles. Ionic and osmotic stresses are brought on by salt stress in plants, and this leads to the accumulation or loss of certain secondary metabolites. In contrast to salt-sensitive plants, salt-tolerant alfalfa plants quickly quadrupled their proline concentration in roots (Petrusa & Winicov's, 1997). Proline accumulation and salt tolerance, however, were found to be correlated in *Lycopersicon esculentum* and *Aegiceras corniculatum*, respectively (Aziz et al., 1998). It was discovered that endogenous JA accumulated in tomato cultivars during salt stress. In general, biotic or abiotic stressors enhance the synthesis and accumulation of polyphenols. Numerous plants have also been shown to contain more polyphenols in various tissues when exposed to increased salt. According to Navarro et al. (2006), red peppers had an elevated total phenolic content and a fairly high salt content. It has been demonstrated that plant polyamines have a role in the way plants react to salt. It was discovered that the levels of free and bound polyamines in the roots of sunflower (*Helianthus annuus* L.) changed as a result of salinity. The examples of salt stress on various secondary metabolites in plant are summarized in Table 15.3.

Influence of Drought Stress Among the most critical environmental stresses affecting plant growth and development are oxidative stress and flavonoids and phenolic acids in willow leaves. Willows grown under drought stress were reported to have increased flavonoid and phenolic acid amounts. Chlorophyll "a" and "b" and carotenoids are affected by drought stress. A reduction in chlorophyll was noticed in cotton under drought stress. Saponins were reported to have lower amounts in *Chenopodium quinoa* plants growing in high water-deficit conditions compared to those growing in low water-deficit conditions. Anthocyanins accumulate in plants under drought stress and at cold temperatures. Plant tissues containing anthocyanins are usually resistant to drought. Anthocyanins are flavonoids that are primarily responsible for shielding plants against drought. For example, chili plants with purple colors withstand drought better than green ones.

Table 15.3 Salt stress on various secondary metabolites in plant

Plant species used	Secondary metabolites	Source
Tomato (*Lycopersicon esculentum* L.)	Sorbitol	Tari et al. (2010)
Sesame (*Sesamum indicum* L.)	GABA	Bor et al. (2009)
Barley (*Hordeum vulgare* L.)	Flavonoids	Ali and Abbas (2003)
Tomato (*Lycopersicon esculentum* L.)	Jasmonic acid	Pedrazani et al. (2003)
Sea rocket (*Cakile maritime*)	Polyphenol	Ksouri et al. (2007)
Prickly burr (*Datura innoxia*)	Tropane alkaloids	Brachet and Cosson (1986)
Silky oak (*Grevillea spec.*)	Anthocyanins	Parida and Das (2005)
Soybean (*Glycine max* L.)	Trigonelline	Cho et al. (1999)
White clover (*Trifolium repens*)	Glycinebetaine	Varshney and Gangwar (1988)
Rice (*Oryza sativa* L.)	Polyamines	Krishnamurthy and Bhagwat (1989)
Wheat (*Triticum aestivum* L.)	Glycine betaine	Krishnamurthy and Bhagwat (1990)
Cenchrus pennisetiformis	Sucrose and starch	Ashraf (1997)

Influence of Heavy Metal Stress Secondary metabolites are also controlled by metal ions (lanthanum, europium, silver, and cadmium) and oxalate (Marschner, 1995). The urease enzyme, which contains nickel (Ni), is crucial to plant growth and requires Ni. Increased Ni concentrations, on the other hand, inhibit plant growth. The anthocyanin levels decrease significantly. Ni has also been established to inhibit anthocyanin accumulation (Hawrylak et al., 2007). Ni has been shown to inhibit accumulation of anthocyanins (Krupa et al., 1996). The concentration of metals (Cr, Fe, Zn, and Mn) produced an oil content of 35% in *Brassica juncea*, which was effective at accumulating metals (Singh & Sinha, 2005). Cu^{2+} and Cd^{2+} have been shown to increase the production of secondary metabolites like shikonin (Mizukami et al., 1977) and digitalin (Ohlsson & Berglund, 1989). Cu^{2+} enhances betalain production in *Beta vulgaris* (Trejo-Tapia et al., 2001). Co^{2+} and Cu^{2+} stimulate the production of betalains in *Beta vulgaris* (Trejo-Tapia et al., 2001).

Influence of Cold Stress The most harmful abiotic stress affecting temperate plants is low temperature. Cold stress boosts phenolic production and subsequent incorporation into the cell wall as suberin or lignin. Cold stress recently has been shown to influence polyamine accumulation. When wheat (*Triticum aestivum* L.) leaves are exposed to a chilly temperature, putrescine (6–9 times) accumulates instead of spermidine, and spermine declines moderately. In addition, alfalfa (*Medicago sativa* L.) produces putrescine under low temperature stress. Cold tolerance is associated with higher amounts of polyamines (agmatine and putrescine), and their amount can be an important indicator of chilling tolerance in seedlings of *P. antiscorbutica*, according to Hummel et al. (2004).

Influence of Light It is known that light is an abiotic factor that affects metabolite production in *Z. officinalis*. It stimulates such secondary metabolites as gingerol and zingiberene in *Z. officinalis* culture when it is combined with UV light. The effect of UV light on anthocyanin accumulation in light-colored sweet cherries was studied by Arakawa et al. (1985). Anthocyanins are synthesized synergistically when UV light with a wavelength of 280–320 nm is combined with red light in apples (Arakawa et al., 1985). The effects of environmental factors such as light intensity, irradiance (continuous irradiance or continuous darkness), and cell biomass yield and anthocyanin production in *Melastoma malabathricum* cultures were investigated by Chan et al. (2010). Moderate light intensity (301–600 lx) resulted in higher anthocyanin levels, while cultures that were exposed to a 10-day period of continuous darkness showed the lowest pigment concentration. Conversely, cultures that were continuously irradiated showed the highest pigment concentration.

Influence of Polyamines In addition to bacteria, plants, and animals, putrescine, spermine, and spermidine are present in a wide variety of organisms (Gill & Tuteja, 2010). Polyamines play an important role in plant development, senescence, and stress responses. Polyamines are present in plants in high quantities and are involved in a variety of physiological processes. Polyamines are present in a wide range of plants and are involved in various physiological processes, including development, senescence, and response to stress. Plants that are tolerant to environmental stresses have higher levels of polyamines than susceptible plants. Polyamine biosynthesis is enhanced in response to environmental stresses in stress-tolerant plants as compared with susceptible plants.

Influence of Plant Growth Regulators Plant organ and tissue cultures have been reported to generate secondary metabolites. Many researchers have tried to increase the productivity of plant tissue cultures by studying hormone-dependent media composition, media composition, and light exposure (Karuppusamy, 2009; Ravishankar & Venkataraman, 1993; Tuteja & Sopory, 2008). Anthocyanin production in plant cell cultures is more productive, with a dry weight yield of up to 20% (Ravishankar & Venkataraman, 1993). 2,4-D, IAA, and NAA, among other cytokinins, promoted growth and anthocyanin synthesis when supplemented at varying concentrations (Narayan et al., 2005; Nozue et al., 1995). Kinetin, which was supplied at 0.1 and 0.2 mg l-1, was the most productive cytokinin. Anthocyanin production and methylation were enhanced when IAA was supplemented at 2.5 mg l-1 and kinetin at 0.2 mg l-1, which was superior to other combinations. Lower concentrations of 2,4-D in the medium limited cell growth and increased both anthocyanin production and methylation (Narayan et al., 2005; Nozue et al., 1995).

Influence of Nutrient Stress When plants are deprived of nutrients, secondary metabolite production may increase because photosynthesis is usually less inhibited than growth, and carbon is allocated predominantly to secondary metabolites. In addition, nutrient deprivation has a significant effect on phenolic levels in plant tissues. Because of the accumulation of phenylpropanoids and lignifications caused by deficiencies in nitrogen and phosphate, osmotic stress resulting from sucrose and other osmatic agents controls anthocyanin production in *Vitis vinifera* cultures.

Influence of Climate Change The level of biodiversity and crop productivity, human and animal health, and well-being in the coming decades will depend on climate change (IPCC, 2007). In the next 50 years, the productivity of cold weather crops such as rye, oats, wheat, and apples is estimated to decrease by 15% (Pimm, 2009). Climate change will cause a decrease in productivity of strawberries by as much as 32% in the next 50 years (Pimm, 2009). In particular, plants are very sensitive to climate change and do not adapt quickly to abnormal conditions. Ozone exposure has been shown to increase conifer phenolic concentrations, whereas low ozone exposure has no effect on monoterpene and resin acid concentrations (Kainulainen et al., 1998). When grown in high CO_2 levels, plants exhibit substantial chemical adjustments. N concentration is lowered in vegetative plant parts, seeds, and grains as a result of lowered protein levels, resulting in decreased protein amounts. In a previous study, elevated CO_2 caused a decline in N concentration in vegetative plant parts, as well as those in seeds and grains, resulting in a decline in the protein level.

15.9 Improving the Production of Secondary Plant Metabolites

The plant secondary metabolite (PSM) synthesized in the plants is very important because of the various health benefits of it in the human body. The major PSMs are phenolic compounds, flavonoids, and anthocyanins. The significant benefits of using phenolics are due to its antioxidant activity, as well as its anti-inflammatory, anticancer, and antitumor properties. These compounds are synthesized with the shikimate pathway in the plant. The accumulation of secondary metabolites usually occurs in plants where they are subject to several stressors and elicitors. Various chemical, physiological, and microbial features act as abiotic or biotic triggers, ultimately leading to increased secondary metabolites (Radman et al., 2003). Drought, salinity, and cold/hot weather are natural conditions that adversely affect crop growth and production (Ramakrishna & Ravishankar, 2011). Elicitors are compounds from abiotic and biotic sources that stimulate plant stress responses, leading to enhanced secondary metabolites or introduction of secondary metabolites (Naik & Al-Khayri, 2016). Various abiotic (salt, light, heavy metals, temperature, drought, etc.) and biotic (proteins, carbohydrates, fungi, arbuscular mycorrhizal fungi) promote the production of the following metabolites (Table 15.4). Several horticultural products such as pears, peaches, mangoes, lychees, onions, apples, hibiscus flowers, green tea, pineapples, and sweet potatoes contain large amounts of antioxidants. Flavonoids are naturally produced in plants. They are phenolic compounds that contribute to their antioxidant capacity. There are some crops with low natural production of flavonoids. In this case, genetic engineering could help improve flavonoid production in crops (Hichri et al., 2011). Anthocyanins are a class of naturally occurring flavonoids found in plants that produce red, purple, and blue colors in

Table 15.4 Biotic and abiotic stresses on the production of various secondary metabolites in plants

Common name	Plant species	Elicitor	Compounds	References
California poppy	*Eschscholzia californica*	Yeast cell	Benzophenanthridine	Farber et al. (2003)
London plane	*Plantanus acerifolia*	Glycoprotein	Coumarin	Alami et al. (1998)
Ashwagandha	*Withania somnifera*	Chitosan	Withaferin A	Gorelick et al. (2015)
Turmeric	*Curcuma longa* L.	Chitosan	Curcumin	Sathiyabama et al. (2016)
Capsicum	*Capsicum annuum*	Cellulase	Capsidol	Patrica et al. (1996)
Opium poppy	*Papaver somniferum*	Chitin	Sanguinarine	Radman et al., 2003
Grape	*Vitis vinifera*	Oligogalacturonic acid	Trans-resveratrol, Viniferin	Taurino et al. (2015)
Plumbago indica	*Plumbago rosea*	Oligogalacturonic acid	Plumbagin	Komaraiah et al. (2003)
Common rue	*Ruta graveolens*	Oligogalacturonic acid	Fluoroquinolone alkaloids	Orlita et al. (2008)
Chinchilla	*Tagetes minuta*	*Pseudomonas fluorescens* and *Azospirillum brasilense*	Monoterpenes, phenolic compounds	Cappellari et al. (2013)
Candy leaf	*Stevia rebaudiana*	*Bacillus polymyxa*, *Pseudomonas putida*, *Azotobacter chroococcum* and *Glomus intraradices*	Stevioside	Vafadar et al. (2014)
Tulsi	*Ocimum basilicum*	*Bacillus subtilis*	α-Terpineol and eugenol	Banchio et al. (2009)
Sweet marjoram	*Origanum majorana* L.	*Pseudomonas fluorescens* and *Bradyrhizobium* sp.	Terpinene-4-o1, cis-sabinene hydrate, trans-sabinene hydrate, and α-terpineol	Banchio et al. (2008)
Pea	*Pisum sativum*	*Pseudomonas aeruginosa* and *Pseudomonas fluorescens*	Phenolic compounds (gallic, cinnamic, ferulic acid)	Bahadur et al. (2007)
Malabar begonia	*Begonia malabarica*	*Glomus mosseae*, *Bacillus coagulans*, and *Trichoderma viride*	Total phenols, orthodihydroxy phenols, tannins, flavonoids, saponins, and alkaloids	Selvaraj et al. (2008)
Cape periwinkle	*Catharanthus roseus*	*Trichoderma viride*	Ajmalicine	Namdeo et al. (2002)

(continued)

Table 15.4 (continued)

Common name	Plant species	Elicitor	Compounds	References
Red sage	*Salvia miltiorrhiza*	*Bacillus cereus*	Tanshinone	Zhao et al. (2010)
Tropical soda apple	*Solanum viarum*	*Glomus aggregatum*, *Bacillus coagulans*, and *Trichoderma harzianum*	Total phenols, orthodihydroxy phenols, tannins, flavonoids, saponins, and alkaloids	Hemashenpagam and Selvaraj (2011)
Common basil	*Ocimum basilicum*	*Aspergillus niger*	Rosmarinic acid	Bais et al. (2002)
Nepal yam	*Dioscorea deltoidea*	*Rhizopus arrhizus*	Steroid (diosgenin)	Rokem et al. (1984)
Common bean	*Phaseolus vulgaris*	*Colletotrichum lindemuthianum*	Krevitone	Dixon et al. (1981)
Turmeric	*Curcuma longa*	*Azotobacter* and *Azospirillum*	Curcumin	Sena and Dass (1998)
Tamarind	*Azadirachta indica*	*Claviceps purpurea*	Azadirachtin	Satdive et al. (2007)
Coleus	*Coleus forskohlii*	*Glomus mosseae* and *Trichoderma viride*	Forskolin	Body and Bagyaraj (2003)
Grape	*Vitis vinifera*	Ethylene, IAA + GA3, and BAP + NAA	Stilbene, resveratrol, anthocyanins	Kin and Kunter (2009) and Qu et al. (2006)
Indian snakeroot	*Rauvolfia serpentina*	BAP + IAA	Serpentine	Salma et al. (2008)
Neem	*Azadirachta indica*	2,4-D	Azadirachtin	Sujanya et al. (2008)
Capsicum	*Capsicum annuum*	2,4-D + GA3, 2,4-D + Kin, and 2,4-D + Kn	Capsaicin	Umamaheswai and Lalitha (2007)
Alexandrian senna	*Cassia acutifolia*	2,4-D + kinetin	Anthraquinones	Nazif et al. (2000)
Beet	*Beta vulgaris*	IAA	Betalain	Taya et al. (1992)
Ashwagandha	*Withania somnifera*	Methyl salicylate	Withaferin A	Gorelick et al. (2015)
Grape	*Vitis vinifera*	Salicylic acid	Stilbene	Xu et al. (2015)
Carrot	*Daucus carota*	Salicylic acid	Chitinase	Muller et al. (1994)
Foxglove	*Digitalis purpurea*	Salicylic acid	Digitoxin	Patil et al. (2013)
Red sage	*Salvia miltiorrhiza*	Salicylic acid	Tanshinones	Xiaolong et al. (2015)
Indian madder	*Rubia cordifolia*	Salicylic acid	Anthraquinone	Bulgakov et al. (2002)
Peppermint	*Mentha piperita*	Jasmonic acid and methyl salicylate	Rosmarinic acid	Krzyzanowska et al. (2012)

Table 15.4 (continued)

Common name	Plant species	Elicitor	Compounds	References
Grape	*Vitis vinifera*	Methyl salicylate and jasmonic acid	Anthocyanin, stilbene, and trans-resveratrol	Xu et al. (2015) and Taurino et al. (2015)
Red raspberry	*Rubus idaeus*	Methyl salicylate	Rubusidaeus ketone benzal acetone	Pedapudi et al. (2000)
Ashwagandha	*Withania somnifera*	Methyl salicylate	Withanolide A, withanone, and withaferin A	Sivanandhan et al. (2013)
Mexican cedar	*Cupressus lusitanica*	Methyl salicylate	β- hujaplicin	Zhao et al. (2001)
Opium poppy	*Papaver somniferum*	Drought stress	Morphine alkaloids	Szabo et al. (2003)
Red sage	*Salvia miltiorrhiza*	Water stress	Salvianolic acid	Liu et al. (2011)
Candy leaf	*Stevia rebaudiana*	Polyethylene glycol	Steviol glycosides	Pratibha et al. (2015)
Salix	*Salix* sp.	Drought	Flavonoids and phenolics	Larson (1988)

flowers, fruits, and leaves and also have antibacterial properties. Health benefits of anthocyanins include protecting coronary arteries, improving vision, and preventing diabetes and obesity. The mechanism of action of anthocyanins is cell signaling-mediated antioxidant activity in mammals. Genetic engineering is used in various crops such as papaya and tomato. Although the anthocyanin content in tomato is very low, a large number of transcription factors and enzymes are involved in anthocyanin biosynthesis. In this regard, anthocyanin-rich transgenic tomatoes are enhanced by highlighting the endogenous ANT1 gene, which helps regulate the binding properties of anthocyanins and extracts, resulting in anthocyanin-rich purple tomatoes. Purple tomatoes were discovered in 2008 by two snapdragon gene compounds, Delilah (Del) and Rosea1 (Ros1), that cause anthocyanin accumulation (Mooney et al., 1995). These genes triple the antioxidant capacity of the tomato fruit, giving the fruit a purple exocarp and mesocarp. Furthermore, feeding tomato-sensitive mice with tomato anthocyanin content was found to extend the life span of the mice, suggesting that these compounds may reduce cancer growth (Giampieri et al., 2018). The transcription factors MYB75 and PAP1, identified in *Arabidopsis*, are involved in anthocyanin regulation (Zuluaga et al., 2008). These genes are introduced into the tomato genome. They have also been shown to independently induce anthocyanin production into tomato plant tissues (Bassolino et al., 2013). Carnation- and rose-transgenic plants probe a dark purple anthocyanin known as delphinidin to synthesize DRF-A and F5’30H from petunia (including the anthocyanin pathway). Transgenic cotton plants producing the Lc gene (a leaf color gene involved in anthocyanin regulation) resulted in purple leaves and reddish anthers and anthers through the accumulation of anthocyanins. Naturally, through the use of genetic engineering and the use of newly developed generic drugs, it is possible to enhance one’s health with the help of professional consumption of fruits and vegetables that have increased levels of metabolites produced in the future. Furthermore, excess phytoene desaturase (ctrI) enzyme in tomatoes showed a 50% decrease in carotenoid content and an almost threefold increase in β-carotene. Furthermore, transgenic tomato plants producing the β-cyclase gene produced up to 60 μg/g new weight μ-carotene in tomato fruit. Plant biotechnology, such as genome editing techniques and second-generation transgenic plants, can play an important role in combating hunger, malnutrition, and certain diseases through the development of nutritionally improved crops. However, more information and policy changes are needed to bring genetically liberated transgenic plants to market and solve the human health problems of our world.

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Chapter 16
Advances in Biofertilizer Production Technologies: Paradigm for Improving Agriculture Through Microbial Resources

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16.1 Introduction

Climate change-related events are mostly to blame for unexpected droughts, torrential downpours, unexpectedly high and abrupt temperatures, snowfall cold and floods (Raza et al., 2019). The reality is that climate change and deteriorating soil conditions coexist on Earth, and we must create a system that is environmentally sustainable while taking into account this coexisting situation (Aggani, 2013). Modern agricultural practices have disrupted the equilibrium between various components, destroyed ecosystems and generated detrimental environmental problems that have impacted soil health and stunted plant growth (Upadhyay et al., 2022a). The excessive use of fertilizers in order to increase yield is the focus of the present trend towards raising agricultural food plummeted, but also the cost of cultivation became very high leading to reduced farming income for farmers. Biofertilizers therefore provide a safe choice for using renewable inputs to improve soil fertility using biological waste with those beneficial microorganisms which impart organic nutrients to the farm produces (Mahmud et al., 2021; Mazid & Khan, 2014). Biofertilizers are best defined as the microbial inoculants or related biologically active formulations containing beneficial microorganism, namely, bacteria, actinomycetes or fungal strains (one or more) in suitable carrier materials for its application for addition, conservation and mobilization of soil nutrients for plants (Mazid et al., 2011). In other words, biofertilizer contains living microbes that colonize the rhizosphere or inside the roots; when administered to soil, plant surfaces or seed, it accelerates growth by making more primary nutrients available to the host plant (Upadhyay et al., 2022b). Biofertilizers have substantial and long-term environmental implications, minimizing the detrimental chemical consequences (Chauhan et al., 2022). At the farm level, the benefits include less soil and water contamination than conventional fertilizers, and even organic manures can be generated to an extent. Biofertilizers therefore provide a safe choice to use renewable inputs to enhance soil fertility by using biological waste with those beneficial microorganisms that generate complementary and synergistic effects with mineral fertilization (Mahmud et al., 2021). The use of biofertilizers has the potential to significantly increase plant nutrient value while decreasing the demand for fertilizer (Upadhyay et al., 2022b). These bio-inputs or bioinoculants, which enhance their growth and yield when supplied to plants, are objects generated using living cells of various types of microorganisms that are capable of mobilizing nutritionally essential elements from unusable form through biological stress (Khan & Naeem, 2011; Upadhyay & Chauhan, 2022; Verma et al., 2022).

A prerequisite for sustainable agriculture is the enrichment of the soil utilizing microorganisms in an attempt to restore the health of the soil and boost its productivity (Singh et al., 2022; Upadhyay & Singh, 2015). The properties of solid, liquid, organic, inorganic, polymeric and encapsulated formulations, nano-formulations and the utilization of microorganisms as biological fertilizers in agriculture are examined.

16.2 Formulations

The process of turning promising goods at commercial inoculants by application of laboratory-proven microbe is known as formulation that may be uses as biofertilizers. Plant growth-promoting rhizobacteria (PGPR)/inoculants can be utilized as microbial formulations made with convenient and affordable carrier materials that include one or more beneficial microorganisms (or species) (Malusa et al., 2012). The generation of high-quality, large-scale biofertilizers is an issue that spans usage and popularity. The choices of a carrier material, as well as its distribution and application procedures, are crucial components in biofertilizer inoculation technology (Itelima et al., 2018).

Characteristics of Good Formulation A good formulation combines viable microorganisms in a suitable carrier material, together with additives that sustain and increase the shelf life of the injected microbe (Bashan, 1998; Lupwayi et al., 2000; Pandya & Saraf, 2010; Sahu & Brahmaprakash, 2016; Sivasakthivelan & Saranraj, 2013; Smith, 1992; Wu et al., 2005; Yadav et al., 2009). The following crucial point should play a significant part in good formulation:

- Stabilize the PGPR or consortia during production, distribution and storage.
- Easy field application methods for application at appropriate time.
- Protection of inoculated microorganism from abiotic and biotic stress, that is, adverse environmental factors at the field conditions.
- Increasing the inoculation organism's ability to reproduce, be viable and interact with the targeted crops once it has been applied. It ought to and should be able to sustain more adjuvant live PGPR cells.
- The microbial formulation needs to have a sufficient shelf life at room temperature or ambient temperature.
- The inoculant formulations should be easily manufactured and scaled up and nutrient supplementation should be possible.
- The inoculant formulations should be economic, affordable and commercially sustainable.
- Formulations should help in improving soil properties and resist pH changes during storage.

- Formulations should help the inoculated microbes to compete with the native soil microorganisms.
- The formulations should have uniform release bioinoculants in entrapped formulation. Formulations with polymer entrapment should be safe and free of dangerous preservatives.
- It can be used with conventional agrochemical equipment for application.
- It ought to meet the international, FCO, BIS and criteria for biofertilizers.

16.3 Types of Formulations

16.3.1 Solid-Based Inoculant Formulations

Solid formulation is a microbial biofertilizer/inoculant preparation in which the microbial inoculum is mixed with an appropriate sterilized solid carrier material in standard proportion (Fig. 16.1). The suitable carrier is the most important part of any formulations and can be defined as "delivery vehicle employed for transferring PGPR microorganism from laboratory to the field" (Sahu & Brahmaprakash, 2016). The carrier material used for formulations is responsible for the delivery of the appropriate count of living cells that are in healthy condition at the time of its application (Fages, 1990). In solid-based formulation, the carrier material has the major portion in terms of volume or weight and allows the PGPR microorganism field applications in good physiological condition (Smith, 1992). In carrier-based formulations, the carrier is the main input used and provides favourable micro-environment

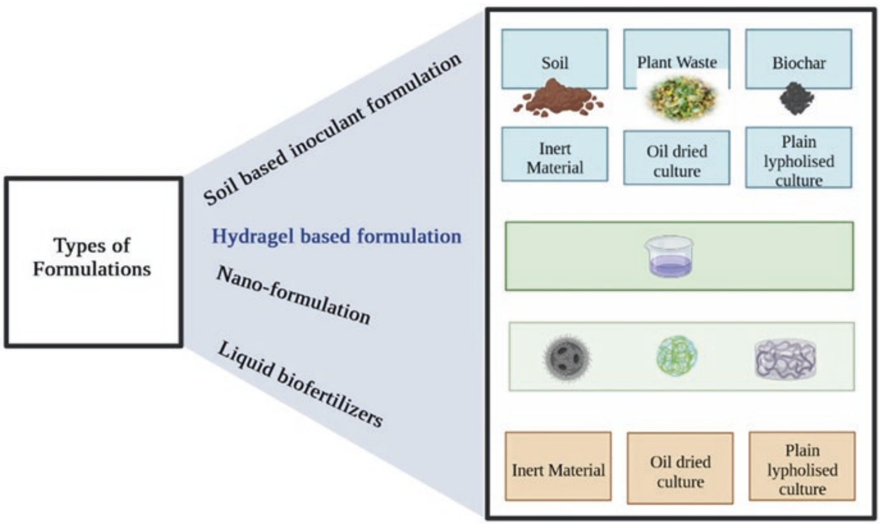

Fig. 16.1 Schematic representation of formulation

to the inoculated microorganism. The shelf life of such products varies based on the carrier material used.

The carrier-based formulation can essentially be easily dispersed or has a good adhesion to stick on the seed surface (seed coating) for its application in root system in rhizosphere (Hegde & Brahmaprakash, 1992). A good carrier material must be economical, easily disinfected (either by autoclaving or gamma-irradiation), have a low tendency to aggregate, be readily available in bulk for large-scale manufacture and have a high buffering capacity to withstand pH fluctuations during formulations (Keyser et al., 1993). The carrier material should also be biodegradable and not cause any environmental pollution upon degradation (Smith, 1992). Survivals of the inoculated microbes are primarily the responsibility of carrier material during the storage as well as post application (Trzcinski et al., 2011).

16.3.2 Characteristics of the Carrier Material Used for the Development of Formulation (Sahu & Brahmaprakash, 2016; Trevors et al., 1992)

1. Excellent water-holding and retention properties.
2. Economic and available in large quantities for industrial production with manageable mixing, curing and packaging operations.
3. Should not promote aggregation or lump during formulation manufacturing process.
4. Should assure the survival of the microbes after its treatment/application on planting material or soil.
5. Should have good adhesion qualities to stick to surface applied.
6. Should be easy to sterilize, nontoxic, biodegradable, chemically and physically uniform insert and should not generate any heat during processing.
7. Should be neutral in pH with good buffering capacity and also allow to amend nutrient supplement as per the requirement.
8. Easy to handle and can be used with standard agro technical machinery.
9. The 10–40 μm size of carrier materials can be used for seed applications, whereas for granular carrier material, 0.5–1.5 mm is advised for soil inoculation.

16.3.3 2 Types of Existing Carriers for Inoculants (Bashan, 1998; Mohammadi, 1994)

(i) **Soils:** these include peat soil, coal powder, fine clays, inorganic soil, etc.
(ii) **Plant waste materials:** agricultural waste material, press mud, vermicompost or composts, farmyard manure (FYM), soybean meal, spent mushroom compost, plant litter or debris, etc.

(iii) **Inert materials:** Perlite, vermiculite, crushed rock phosphate, calcium sulphate, polyacrylamide gels and alginate beads are some of the materials used.
(iv) **Biochar.**
(v) **Plain lyophilized microbial cultures and oil-dried bacteria:** plant growth-promoting inoculants can also be stored for longer period by using freeze-drying or lyophilization, which allows achieving high survival rates, using a cryoprotectant, namely, mannitol, sucrose, casein, trehalose, etc. Lyophilized microbial cultures can be used directly or also can be encapsulated or mixed with a carrier.

16.3.4 Forms of Carriers (Bashan, 1998; Ciafardini & Barbieri, 1987; Smith, 1992)

- Powder (75 μm–0.25 mm).
- Granular particles (100–200 μm in diameter).
- Beads (a diameter range of 3–4 mm).

The powder-type formulations can be used directly for seed coating or soil applications suspended in a liquid containing suitable sticker material (e.g. gum, CMC, etc.) to form slurry for seed coating or root dipping applications.

16.4 The Carrier Material Classified Based on Its Source

Natural Carriers Peat soil has been the most commonly and widely used natural carrier material for microbial inoculant preparation, since all the initial *Rhizobium*-based biofertilizers were based on peat soil (Smith, 1995). Peat soil maintains the metabolic activity of inoculated microbes; sometimes the inoculated microorganism may also multiply; however, the disadvantage of peat is its inconsistent quality since the peat is made from different inputs and secondly the acidity of peat soil which adversely affects the viability of inoculated PGPR (Corich et al., 1996). Peat soil had very high number of microbes, and sometimes these microbes compete with the inoculated PGPR and reduce the viability of inoculated PGPR (Van Elsas & Heijnen, 1990). The plant waste materials are also the alternative choice to be used as carrier; however, they also have the similar disadvantages associated with peat soil.

In the recent times, with more awareness on organic farming, the different types of compost are becoming the preferred choice of natural carriers (Upadhyay & Chauhan, 2022; Vassileva et al., 2010). Kumar et al. (2010) inferred that the inoculation of N-fixing and P-solubilizing PGP strains enhanced the vermicompost quality, thereby resulting in enhanced levels of N and P in the resultant final product. However, the compost is not a preferred choice for arbuscular mycorrhizal since the mycorrhization rate is reduced on supplementing with cellulose-rich amendments even when cellulose can increase the asymbiotic hyphal growth of AMF (Gryndler et al., 2002). Sawdust was also used as a carrier for various strains of bacteria (Arora et al., 2008). With more emphasis on carbon sequestration, abundant availability of sludge was also considered as natural carrier. Sludge-based carriers are having neutral pH with good water-holding capacity and provide good shelf life (130 days); however, the drawbacks with sludge are that heavy metal and other toxic contaminates may pollute the soil. Carriers for different formulations may also comprise coal, clays, inorganic soils (lignite, lapillus, volcanic pumice or diatomite earths), vermiculite, perlite, bentonite, etc., in different combinations (Millner & Kitt, 1992; Smith, 1995).

16.5 Polymer-Based Carriers

The additives added into the microbial inoculant formulations protect the microbial cells on seed and in the soil not only from high temperature but also due to desiccation (Biradar & Santhosh, 2018). Due to their high water retention capacities, capacity to reduce heat transfer and favourable rheological qualities, several polymers have been employed to produce inoculants (Table 16.1).

Alginate and carrageenan are both the most frequently used polymer-forming ingredients in microbial formulations that are applied to rhizospheric systems, despite the fact that water-soluble polymeric materials like agar, methoxy pectin, gellan gum and mixtures of xanthan and locust bean gum are also frequently used in the development of microbial-based products (Bashan, 1998; Vassilev et al., 2001, 2005, 2014, 2020). The natural polymer of D-mannuronic acid and L-glucuronic acid known as alginate, which is generated from brown macroalgae like *Sargassum sinicola* and *Macrocystis pyrifera* (kelp), is frequently used for the encapsulation of microbial inoculants (Yabur & Bashan, 2007) (Table 16.2).

When the alginate solution is added to the cation solution, beads develop because the alginate polymer combines with multivalent cations (such as Ca^{2+}) to create a dense three-dimensional lattice with pores that are typically between 0.005 and 0.2 mm in diameter (Smidsrod & Skjak-Braek, 1990). Alginate beads typically have a diameter of 2–3 mm; however, smaller micro-beads that can hold 108–109 CFU g^{-1} have also been proposed (Bashan et al., 2002). Alginate beads can keep enough live cells alive for several months (Trzcinski et al., 2011; Van Veen et al., 1997). Formulation is required for an additional treatment during sowing in order for the bacteria to move through the soil and get close to the plants (Fig. 16.2).

Table 16.1 Encapsulation with other polymer materials

Material	Advantages	Limitations	References
K-Carrageenan	Comparing the cell density of the beads to the original bacterial culture, it may be possible to get a higher cell density	Bacteria could be killed by beads formed at high temperatures	Wada et al. (1980) and Nasri et al. (1987)
Polyacrylamide	Easily available as well as expensive	Limited degradability along with toxic nature of the monomers makes it a liability for the environment	Dommergues et al. (1979) and Keller and Lingens (1984)
Agar agarose	Easily available	It is incredibly expensive and requires high temperature for solidifications and degradation is slow	Bashan et al. (1982)
Xanthan-carbo gum	Provides good protection for bacteria	Unknown	Jung et al. (1982) and Mugnier and Jung (1985)
Polyurethane foam	–	–	Briglia et al. (1990)
Flocs of bacteria	–	–	Neyra et al. (1995)
Polysaccharide adhesive	–	–	Berge et al. (1990)
Chitin Chitosan	Biodegradability, nontoxicity, simplicity of modification and antibacterial activity	Expensive	Chanratana et al. (2018)

Table 16.2 Other carrier materials used for biofertilizers

Carrier material	Bacterium inoculant	Characteristics	References
Oxalic acid industrial waste	*Rhizobium*	Seed inoculation Rhizobium proliferation in a carrier at room temperature for up to 90 days Grain yield, nodule count and nitrogen content all increased significantly as a result of carrier sterilization	Kaushal et al. (1996)
Alginate-perlite dry ganule	*Rhizobium*	Soil inoculation After 180 days, *rhizobium* bacteria continued to thrive in dry granules Dry storage of the inoculant is possible without significantly reducing its viability	Hegde and Brahmaprakash (1992)

(continued)

Table 16.2 (continued)

Carrier material	Bacterium inoculant	Characteristics	References
Composted sawdust	*Bradyrhizobium*, *Rhizobium* and *Azospirillum*	Seed inoculation The inoculant strains have grown and survived well	Kostov and Lynch (1998)
Agriperlite, expanded clay, kaolin, celite, diatom, porosil MP, microcel and vermiculite	*Agrobacterium radiobacter* K84	Crown gall control Screening was done to identify a better K84 cell formulation The impact of carrier water content and storage temperature on K84 survivability was investigated	Pesenti-Barili et al. (1991)
Cheese whey-grown cells in peat	*Rhizobium meliloti*	Seed inoculation Improved life at different temperatures during storage, including desiccation	Bissonnette and Lalande (1988)
Mineral soils	*Rhizobium*	Seed inoculants Rhizobium performed significantly better at 4 °C than at higher temperatures	Chao and Alexander (1984)
Coa	*Rhizobium*	Seed inoculants The growth and survival of *R. phaseoli* strains were supported by seven out of the eight coals studied. After a year, the majority still had more than 107 rhizobia per g	Paczkowski and Berryhill (1979)
Granular inoculants amended with nutrients	*Bradyrhizobium japonicum*	Soil inoculants Bentonite, illite and smectite or silica granules altered with glycerol, Na glutamate and *Bradyrhizobium japonicum* inoculants or both Improved early soybean nodulation and increased grain N content	Fouilleux et al. (1996)
Soybean oil or peanut oil added with lyophilized cells	*Rhizobium*	Seed inoculants When rhizobia are inoculated on seeds and exposed to circumstances of dryness and high temperature, they offer higher protection than inoculants based on peat	Kremer and Peterson (1982)

(continued)

Table 16.2 (continued)

Carrier material	Bacterium inoculant	Characteristics	References
Perlite	*Rhizobium*, *Bradyrhizobium* and *Bacillus*	Seed inoculants A sucrose adhesive combined with a perlite carrier improved the survival of bacteria on seeds Generated a comparable amount of nodules, nodule dry weight, crop yield and nitrogen content as peat-based inoculants. Carrier gave better survival of bacteria on seeds	Daza et al. (2000)
Wastewater sludge	*Sinorhizobium meliloti*	Seed inoculants Sludge had the same or a higher capability than peat to sustain *S. meliloti*'s life, which demonstrated the appropriateness of employing it as a carrier	Reban (2002)
Wheat bran, sugarcane baggase	*Rhizobium*/*Bradyrhizobium* and rock-phosphate-solubilizing fungus *Aspergillus niger*	Soil inoculants Peat has the most cultured microorganisms, followed by bran and sugarcane baggase	Abd-Alla and Omar (2001)
Nutrient-supplemented pumice	*Rhizobium*	Seed inoculants Excellent handling and storage qualities; while sowing, it might be combined directly with the seeds	Einarsson et al. (1993)

16.6 Biochar

Through pyrolysis, hydrothermal carbonization, gasification and torrefaction, a carbon-rich substance known as "biochar" is produced (Lehmann, 2007). It is favourable for the development of bacteria and fungi due to its porosity and adsorption capacity, which allows it to be used effectively as a carrier to microbial inoculants or PGPR (Upadhyay et al., 2022b). Another benefit of employing biochar as a carrier is that it is relatively resistant to microbial deterioration and that carbon dioxide (CO_2) from terrestrial organic carbon returns to the atmosphere more gradually, making it an efficient carbon sequester (Rawat et al., 2019).

O/C ratio 0.4, H/C ratio 0.6, surface area > 100 m^2g^{-1}, black carbon >15% C and polyaromatic hydrocarbons below the level defined by national or international

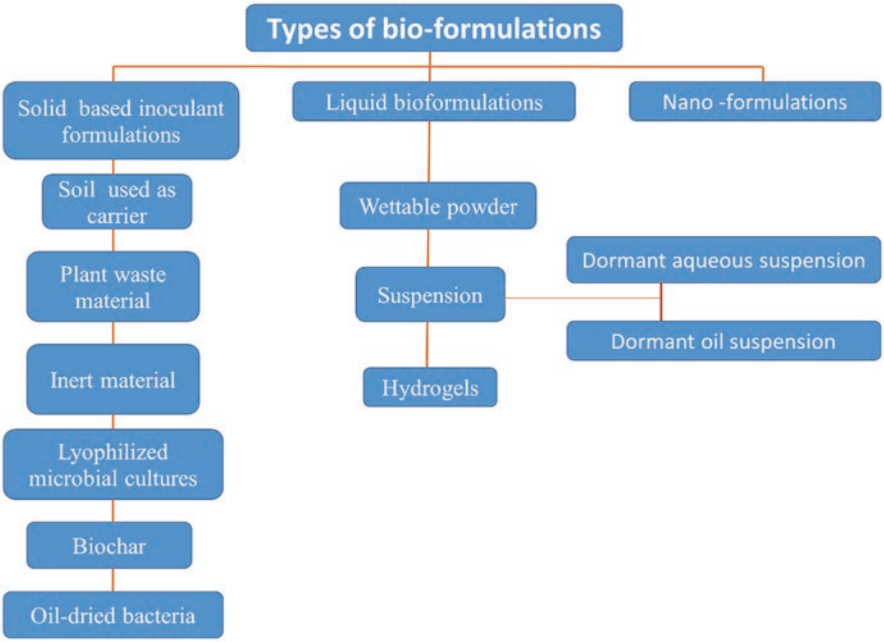

Fig. 16.2 Schematic representation of bio-formulation

legislation are recommended for biochar to be used as a carrier for microbial inoculants (Schimmelpfennig & Glaser, 2012). The use of biochar as a vehicle to immobilize PGPR was detailed by Ajeng et al. (2020), who also described the potential mechanisms of inoculants based on biochar that might be used to achieve agricultural sustainability. In comparison to other carrier materials, the PGPR that included biochar was also shown to be effective, according to El-Hadidy (2019). It is possible that employing biochar as an inoculant carrier also enhances the microbial network in the amended soil since biochar-based inoculants encourage root colonization by the injected arbuscular mycorrhiza fungi (Saxena et al., 2013). On the other hand, adding biochar may improve the soil's physicochemical properties and capacity to retain water, which would nourish the local soil microbial community and subtly promote plant growth.

16.7 Disadvantages of Biochar as Carrier

1. Biochar as an inoculant carrier may cause a shift in the soil microbial communities (Jeffery et al., 2017).
2. Scaling up to an industrial scale may be difficult.
3. Immobilization of PGPR into biochar for formulation development may cause diffusional problems and a decrease in the microbial activity due to the formation of the matrix (Trelles & Rivero, 2013).

4. The porosity of biochar is compromised due to the PGPR immobilization, namely, spore formation, organic acid and biofilm formation, volatile organic compounds formation, etc., ultimately reducing its properties as an absorbent.
5. Due to increasing application of biochar in the soil, smaller biochar particulates or nano-biochar will also be accumulated in the soil which might be toxic to soil microflora and also pollute the soil and water (Liu et al., 2018).

16.8 Advantages of Carrier-Based Biofertilizer Formulation (Cbbf)

- It has been demonstrated that increased biological breakdown of organic pollutants, increased nutrient availability to plants and tolerance to soil-borne plant diseases are all present (Warren et al., 2009).

16.9 Disadvantages of Cbbf

- Shelf life of microbes is only 6 months.
- It cannot withstand UV light or temperatures above 30°.
- At the time of manufacturing, there were only 10^8 cfu/ml of these microorganisms. Every day, this number drops. At the end of the 4 months, it drops to 10^6 CFU/ml, and at the end of 6 months, it is almost negligible (Santosh, 2015).
- The carrier-based biofertilizers are not effective due to poor quality, high contamination and unpredictable field performance and thus are less popular among the farmers.

16.10 Liquid Biofertilizers

Liquid formulations are sprayable propagules of microbial agents or a consortium of microorganisms, protestants or substances suspended in appropriate liquid medium that facilitate the development of dormant spores or cysts for a longer shelf life and resistance to unfavourable conditions that help to increase the biological function of the target site.

These have been primarily classified into the following:

- Wettable powder formulations (dusts, granules and briquettes).
- Suspensions (oil- or water-based and emulsions).
- Hydrogel-based liquid formulations.

16.11 Wettable Powder Formulations

This category includes wettable dry powders that need to be activated right before distribution using a liquid or water carrier. Based on particle or aggregate size, these formulations mix inoculums in a semidry state, such as dusts, granules and briquettes (Pindi & Satyanarayana, 2012). It is necessary to use low absorbance inert diluents as carriers since dusts typically range in size from 5 to 20 mm. The target site benefits from low dust particles or those smaller than 10 mm; however, they are hazardous to breathe in (Goenadi et al., 2018). Organism suspensions, which make up around 30% of the dry weight of dusts, are commonly prepared in a blender. Typically, these suspensions are created by introducing the organism into an airstream.

Pellets are larger than 10 mm^3, briquettes are huge blocks of up to several cubic centimetres and granules are discrete masses between 5 and 10 mm^3. These materials, like dusts, contain inert carriers such as clay minerals, starch polymers and pulverized plant residues that hold the organisms (Pal et al., 2015). The main factors considered when selecting a good carrier include absorption (particularly important for creating slurries of organisms), hardness, bulk density and liquid dispersion rate in water, among others. Soft carriers, like bentonite, exhaust quickly to release the organism. The substance is coated with a variety of compounds to delay or limit the release rate, which frequently varies with the rate of release and the unit size. In granules, there are typically 20–30% organisms present. In order to make wettable formulations stable on shelves during storage and easily adhere to seeds, 3% gum is added (Pindi & Satyanarayana, 2012).

16.12 Dormant Aqueous Suspensions

For the long-term viability, they use growth inhibitors, contaminant inhibitors, such as sodium azide, sodium benzoate, butanol, acetone, fungicides, insecticides, etc. After 8 months, the nodule size is reduced and the nitrogen fixation process is impacted when the *Rhizobium* dormant liquid formulations are utilized. This is because a lengthy activation period is required. It has been noted that the extreme dormant state has been passed by the bacteria *Azospirillum* and *Azotobacter* and phosphate-solubilizing microbes. As a result, they require a significant reactivation time after application to crops. For short-lived crops, this prolonged period is undesirable.

16.13 Dormant Oil Suspension

Oil is the ideal medium for supplying inoculants in a viable state. High concentrations of microorganisms in varying states of dehydration can be suspended in oil and still remain viable. Once applied, the oil prevents the water from evaporating by trapping it around the organism. This is especially beneficial for organisms that are sensitive to desiccation or when used for horticultural crops with irrigation systems. The addition of chemicals to the oil and/or aqueous phases of water-in-oil emulsions may enhance both release kinetics and cell survival. Due to the physiological dormancy of the organisms delivered by this formulation, it prevents contaminants from growing while it is being stored. In order to create inoculants with a long shelf life, the bacteria/fungi were successfully dried by continuous aeration as a suspension in oil. The particle settling rate and viscosity are about comparable. Utilizing colloidal clays, polysaccharide gums, starch, cellulose or synthetic polymers can accomplish.

Frequently used additives include stabilizers, adhesives, nutritive substances and protectors (Bashan et al., 2014). A wide range of chemically varied compounds, including sugars, polymers and amino acids, are included on the long list of putative protective agents.

Due to its protective effect, which is known as the "water replacement hypothesis", which aids in maintaining the membrane fluidity and thus integrity, trehalose is one of the most researched desiccation protectants (Leslie et al., 1995). Trehalose has a good protective function, but because of its high price, it cannot be cheaply packaged in vials. Skim milk, liquid growth medium, horse serum (Peiren et al., 2015), ficoll, hydroxyethyl-cellulose, hydroxypropyl methyl-cellulose, polyvinyl alcohol (Wessman et al., 2011), glucose, sucrose, maltodextrin, fructose, lactose, sodium glutamate, cysteine, dextran, polyethylene glycol and glycerol (Costa et al., 2000) are additional possible protective agents.

16.14 Various Benefits Over Conventional Carrier-Based Biofertilizers

- Extended life span.
- Absence of defilement and thus increase in enzymatic activity.
- Prevention of properties' storage at up to 45 °C.
- Highly competent over native population.
- More than 109 cells/ml populations can be maintained for 12–24 months.
- Simple recognition because of the characteristic smell of fermentation.
- Savings on binding agent, comminution, neutralization, sterilization, prepping and conveyance.
- Quality assurance procedures are simple and rapid.
- More effective soil and seed and seed survivability.
- There is no need to keep biofertilizer producing facilities running all year.
- Extremely user-friendly for farmers.
- The dosage is ten times lower than that of powder biofertilizers.
- Generous business profits.
- Excellent export prospects.

Table 16.3 Applications for hydrogel and its features (Suman et al., 2016)

S. No.	Attribute	Applications
1	Chemical composition	Cross-linked anionic polyacrylate
2	Appearance	Amorphous granules
3	Size	20–100 mesh (microgranules)
4	pH	7.0–7.5
5	Stability at 50 °C	Stable
6	Sensitivity of UV light	Insensitive
7	Temperature	40–50 °C
8	Stability	~2 years
9	Other properties	Absorbs 400 times its dry weight in water and eventually discharges the same amount
		Maximal absorbency is shown at temperatures (40–500 °C), a property of arid and semiarid soils
		Increases the rate of seed emergence and germination by %
		Minimizes the need for fertigation, irrigation and the amount of urea to be administered

16.15 Hydrogel

The chemical stabilization of hydrophilic polymers in a three-dimensional network with water as the dispersion stage results in the production of a specific class of macromolecular gels known as "hydrogels" (Narjary et al., 2012). Superabsorbent hydrogel (Table 16.3) is generally commonly used as the absorbent core material (Buchholz & Peppas, 1994). Super absorbents have structures that are 100% normal and have no environmental damage. Water availability for plants has been demonstrated to increase when hydrogel is applied to sandy soils by limiting drainage holes and increasing retention pores while lowering saturated hydraulic conductivity. Hydrogel-based biofertilizers have been designed to rectify the drawbacks of liquid- and lignite-based biofertilizers, which will be the only available substitute for efficient, sustainable agriculture. To mitigate crises such as soil degradation, recurring droughts or food security, bioinoculants based on hydrogel extend the shelf life of the bioinoculants, which requires an understanding of their actions and performances in the soil.

16.16 Nano-Formulations

Utilizing nano-biotechnology opens up new opportunities for the creation of carrier-based microbial inocula (Navrotsky, 2000; Parashar et al., 2008). Inorganic or organic nanoparticles with one or more sizes of the order of 100 nm or less are used in nanotechnology (Auffan et al., 2009). Hybrid systems, having applications in many fields, including agriculture, are created by combining entire cells with nanostructures (Bailey et al., 2010). Actually, filters made of radially aligned carbon nanotube walls that can absorb *Escherichia coli* at a scale smaller than that of cells, called nanoscale constructs, were created (Srivastava et al., 2004). The same approach might potentially be used to collect bacterial cells from fermentation processes and transport them to the plant. Because of their large surface area, ease of synthesis and cost-effectiveness, nanotubes can be used to produce biofertilizers with a greater degree due to their physical stability. The durability of biostimulators and biofertilizers may be improved by the use of nano-formulations in terms of desiccation, heat and UV inactivation. The mycelium desiccation was decreased by the addition of 7–14 nm hydrophobic silica nanoparticles to the water-in-oil emulsion formulation of the *Lagenidium giganteum* biopesticide fungus. Improvements were made to the formulation's physical properties, and even after 12 weeks of storage, the microorganism was still effective at room temperature (Vandergheynst et al., 2007).

16.17 Impact of the Applications of Various Microbial Formulations on Agriculture in India

The usage of biofertilizers is rising dramatically in India because of a large number of regional suppliers who supply relatively small farms. India produces the bulk of the world's biofertilizers, with the south accounting for over 55% of production, the west for 24.7%, the north for 15.2% and the east for 5% (Praveen & Singh, 2019). Because of the expanding demand for biofertilizers on the international market, growing awareness of the risks and negative effects of chemical fertilizers and air pollution and developing tendencies for organic food items, the use of biofertilizers has expanded significantly, as shown in Fig. 16.3. The USA and Canada are the two countries driving the regional market for biofertilizers. In contrast to Europe, which is the second-largest consumer of biofertilizers and held a 30% share of the

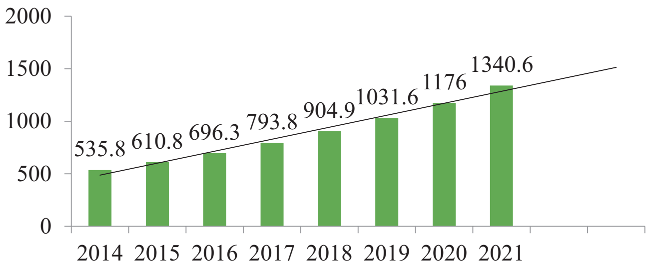

Fig. 16.3 At a CAGR of 14.0% from 2015 to 2020, the global biofertilizer market is anticipated to reach USD 1.88 billion by that year. The market as a whole was worth $440.0 million USD in 2012. From 2015 to 2020, the market for biofertilizers is anticipated to expand at a CAGR of 14.0%. (Source: Expert interviews and market and market analysis)

Table 16.4 Indian biofertilizer market status and growth in comparison to wealthy nations (USA)

India	USA
Noncompetitive market	Competitive market
Demand is less	More demand due to growing environmental concerns
Many small farmers and medium local companies	The inoculant market is dominated by large multinational companies: Novozymes and BASF
Details on the content of the products sold are often limited or unavailable	Details are well labelled

worldwide biofertilizer market in 2019, the USA accounted for 32.6% of global biofertilizer revenues in 2019. Growers' trust in the products is increasing as a result of recent regulatory changes. The Chinese government supports the use of biofertilizers while acknowledging the need for advancements in technology, product quality and product promotion. Africa is still expanding its inoculant market, but it is constrained by farmers' ignorance, inadequate logistics and poor distribution. By looking at Table 16.4, it is possible to assess the current state of biofertilizers in India and the market's expansion.

16.18 Conclusion

Understanding the many circumstances and related characteristics in the soil-plant-microorganism system is crucial to increase the efficacy of PGPM inoculum applications in the field. Actually, a number of factors, particularly in the case of bacteria, affect how rhizocompetent they are. Numerous environmental factors, such as soil pH, mineral nutrients and water content, plant species, genotype and physiological status and the presence of other microbial species, in addition to the genotype and physiological status of the inoculated strain, affect the size and makeup of the populations supported by the rhizosphere (Albareda et al., 2006a, b; Upadhyay et al., 2022b). Most of the treatments chosen thus far are meant for annual crops (mainly legumes, cereals and some vegetables). However, there is an increasing demand from other agricultural productions, particularly organic farming and integrated production systems, where the use of synthetic inputs is neither prohibited by law nor constrained by it. These other agricultural productions include the production of fruits and vegetables. In soilless and covered crops, where the use of inert substrates and managed growing conditions should increase the predictability of PGPM applications relative to open fields, commercial inoculants can find a substantial market. It will be feasible to expedite the transition of agriculture to more environmentally friendly production methods while simultaneously enlarging the inocula market by selecting certain strains for each of these crops. However, the development of technologies geared towards enhancing inoculum delivery, perhaps by redesigning sprinklers and sprayers widely used for irrigation and plant defence, may further boost the usage of the inoculum and raise the efficacy of PGPM applications in agro-ecosystems.

16.19 Future Prospects

Attempts to lessen abiotic stress conditions in crops (such as drought, salinity and inorganic and organic pollutants), as well as to enhance food quality, are linked to potential difficulties in selecting PGPM. Further study is needed on a number of topics, including improvements of the production process for microbial inocula consortia, the development of new nanoparticle-based carriers, the optimization of application devices and the application time for polyannual crops, in order to broaden the application and effective utilization of PGPM in agriculture.

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Correction to: Nano-Biofortifed Crop Plants with Zinc for Human Health

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Index

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