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Nano Fertilizers to Enrich Plant Protein – A Way to Improve Protein Content in Plant-Based Meat

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Abstract:

In the growing era of agro-science, nanotechnology helps to upgrade agricultural production by increasing the efficiency of inputs and minimizing relevant losses. The use of nanoparticles enhance food quality and safety, reduce agricultural inputs and enrich crops by absorbing nano-scale nutrients from the soil and acting as nanocarriers. Nanomaterials are used to coat nitro-fertilizers which are delivered to the crops via hydroponic systems, the soil, or pre-soak strategies. Compounds like Ag, Fe, Cu, SiO_2, Zn, ZnO, CeO_2, and carbon nanotubes have unique physicochemical properties such as influencing the uptake, translocation, and accumulation of nutrients that inherently enhance plant growth and stress tolerance. Nanoparticles can play a role in each stage of plant growth-during seed germination, root and shoot growth, biomass, and fibrillation of plant proteins forming a hybrid nanocomposite. Nanomaterials can penetrate the seed layer and induce the ability of absorption and utilization of water, which stimulates the enzymatic system. Nanomaterials such as ZnO, TiO_2, carbon nanotubes, and FeO are reported to enhance the quality of many crops including peanut, soybean, mungbean, wheat, onion, spinach, tomato, potato, and mustard. This chapter aims to understand the nano fertilizer's effect on the improvement of the plant quality that would, in turn, enhance the plant protein which helps to create better plant-based meat. To produce plant-based meat, plant proteins from different sources are used but due to the factors such as availability, cost, and processing functionality, soy and pea proteins are widely used as the main ingredients. Both consist of an albumin fraction and a globulin fraction.

Nano fertilizers enhance the amount of albumin during seed germination and improve salt tolerance in these plants so that more amount of globulin can be extracted. Thus the protein levels are increased in the soy and pea plant which in turn helps to improve the plant-based protein meat which could compete with animal meat products.

Keywords: Nano fertilizers, plant protein, plant-based meat, nanoparticles, nanomaterials

1. Introduction

Nanotechnology is a field of science related to building materials in nanometers and used to help in improving the agriculture and healthcare sector; especially in agriculture, it has significant potentials to improve food quality around the world [1]. Many novel nanoparticles and nanodevices are expected to be used in farms to improve crop yield. Nanoscale, nanoscale coating, and nanoscale additive are three types of nano fertilizers. Nanoscale fertilizers are made of nanoparticles with nutrients, whereas nanoscale additive fertilizers are made with nanoscale additives, and nanoscale coating fertilizers are loaded with nanoparticles prepared using chemical (bottom-up) or physical (top-down) approaches. The nutrients are either coated with a thin polymer film particle or encapsulated in nanoporous materials. Encapsulation with microbes namely fungi or bacteria improves plant growth by increasing the potassium, nitrogen, and phosphorous in the root zone. Based on the mode of action the nano fertilizers are divided as nanocomposite fertilizers, control loss fertilizers, control or slow-release fertilizers, and magnetic fertilizers [2]. The nanomaterials used vary in mechanism and properties. The porous nanomaterials help in reducing nitrogen loss by enhancing the plant uptake process and regulating demand-based release, whereas ammonium-charged zeolites enhance the solubility of phosphate minerals, and graphene oxide film aids the release of potassium nitrate by minimizing losses by leaching and extending the time [8]. Nano-calcite with nano Fe_2O_3, SiO_2, and MgO improves iron, calcium, and magnesium uptake; especially it enhances phosphorus intake with micronutrients such as manganese and zinc [3].

Studies on nano fertilizers have revealed that nanomaterials can penetrate the seed's coat and induce water utilization and absorption ability which stimulates the enzymatic function of plants and changes the quality of the plant germination and seedling. Peanut, soybean, mung bean, mustard, tomato, wheat, potato, onion, and spinach are some of the examples [4]. Nano-zinc and boron fertilizers help improve fruit yield and quality without hampering any physical characteristics. Thus using nano fertilizer in agriculture can cause an evolution in agri-based alternatives as it leads to high-quality plants and the plant proteins from such plants can be used to create plant-based meat as a substitute for animal meat.

Plant-based meat is an alternative form of animal meat made from plants that taste, appear, and are cooked like conventional meat. Recent studies, on conventional meat, have proved that consumption of red meat is connected to problems such as heart disease, cancer, and diabetes [5]. Thus, plant-based meat is a healthier alternative compared to animal meat. Considering the health and environmental benefits, consumption of plant-based meat is expected to grow exponentially and the improvement in their quality and nutritive contents is a need of the hour which can be achieved by using nano fertilizers [6].

Plant products that are used to mimic animal meat have to satisfy nutrition, taste, smell, appearance, and mouthfeel. Plant-based meat focuses mainly on the protein and the oil contents in the plants, the seeds, or in the fruits. Some of the plant products widely being used are soy, wheat, chickpeas, fava beans, and other legumes, because of their easy availability and protein content. Nano fertilizers have been proved to enhance the nutritional content of the plant. Seed priming using Zn, Fe, and Ca nanoparticles has increased seed weight and grain yield in chickpeas [7]. Iron oxide and sulfate nanoparticles could increase the biomass content in wheat which is another key raw material for plant-based meats.

This chapter discusses the nano fertilizers which play a vital role in benefiting plants by increasing protein content, growth rate, yield rate, and metabolism. If the plant and plant products get upgraded to a better quality with a higher nutritive coefficient then the plant-based meat out of them will be highly nutritive enough to compete with the animal-based meat.

2. Strategies to improve the quality of plant-based meat

Plant-based meat is an alternative source of conventional animal meat that mimics the organoleptic properties and the nutrient values. Furthermore, it also mimics the appearance, aroma, flavor, and texture of animal meat by manipulating structure, color, and fat marbling. Skeletal muscle, adipose tissue, supporting cell types, vasculature networks, and connective tissues are the major components of animal-based protein and need to be mimicked [6].

In the 1980s a synthetic meat flavor was developed which is composed of fat, amino acids, glycoprotein, sugar, salt, nucleotides, monosodium glutamate and determined by a sensory panel to be equal or superior to meat extract. Plant-based sulfur meat's texture can be influenced by mycelium cultivation, high-moisture extrusion, mycelium cultivation, 3D printing, and shear cell technology [11]. In some plants, few compounds have to be removed during post-harvesting due to the astringent taste and bitterness of plant proteins. For example, lipoxygenase, isoflavone, and saponin present in soy products have beany, strong grassy, and bitter flavor which can be reduced through germination or heating [10].

Table 1 Nano fertilizers and their effects on plants and the soil ecosystem

Nano Fertilizers	Benefits	Drawbacks	References
Nitrogen-zeolite nanocarriers	Enhance energy metabolism and protein synthesis. Helps in plant growth, chlorophyll generation, and photosynthesis.	Accumulation of nitrogen attracts pests and diseases. Reduced stem strength leading to lodging while flowering and grain filling	[12], [16]
Phosphorous-nano hydroxyapatite fertilizer	Increase growth rate in soybeans by 32.6% Increase seed yield in soybeans by 20.4%	Not very effective in high phosphorous absorbing soils	[15]

Surface modified phosphorous-zeolite nanocarriers	Increases phosphorous usage efficiencies in plants, which conventionally doesn't exceed 20%	Long term use affects soil pH Possible toxic effects of toxic-metal immobilization by zeolite	[15], [16]
Nano calcium fertilizers	Increase in fruit yield compared to conventional $CaCl_2$	Increase in uptake results in disturbed ion balance, cytosol pH, and solubility of certain ions (E.g.: Fe)	[15], [13]
$Mg(OH)_2$- nanoparticles (500 ppm)	Increase in plant growth and seed germination in *Zea mays*	An increase in magnesium concentration can decrease the amounts of carotenoids	[12]
Combined Mg and Fe nano fertilizers	Increase in the yield of black-eyed peas	Decrease in amounts of carotenoids. Possible iron toxicity, resulting in lowered soil pH	[12]
Green synthesized sulphur nanoparticles (using *Ocimum*	Decrease in Mn uptake, limiting Mn toxicity. Enhanced sulfur metabolism.	Sulfur toxicity results in increased concentrations of salts, and pH imbalance	[12]

***basilicum* leaf extract)**	Increased water content in seedlings.		
Iron-chelate nano fertilizers	Stable and slow release of iron in a broad range of pH	Iron toxicity resulting in lowered soil pH	[12]
Foliar application of Fe Np (500 mg/L)	Increase in number of pods by 47%, seed weight by 7%, Fe content in leaves by 34%, and chlorophyll content by 10%	An increase in iron content in leaves leads to leaf bronzing.	[12]
Fe_2O_3 nanoparticles	Increase in protein content in various plant species	Reduces root oxidation power	[15]
Ferrous oxide nanoparticles	Concentration at <0.75 g/L increase chlorine, lipid, and protein content in soybeans	Higher concentrations (0.75-1 g/L) decrease protein and lipid contents	[15]
Zero-valent iron (nZVI) nanoparticles	Increase in chlorophyll content in 20-days old *Medicago sativa* seedlings	Decrease in carbohydrate (glucose, xylose, and lignin) was observed in nZVI treated soil	[12]

Nano-zinc oxides	Protects plant from UV radiation Helps in the recovery of nutrients in the soil.	An increase in concentrations leads to anomalies in seedling biomass, root growth, and seed germination.	[16], [12]
Copper nanoparticles	Improve stress tolerance in wheat leading to an increase in proteins concentration involved in glycolysis and starch degradation	Leaf discoloration, stunted growth, and cell death at higher concentrations	[12], [14]
Biogenic copper nanoparticles	Increase in weight, height, and root length of pigeon pea	Leaf discoloration, stunted growth, and cell death at higher concentrations	[14]
Cu-chitosan nanoparticle foliar spray	Improvement in yield and growth in finger millets	Leaf discoloration, stunted growth, and cell death at higher concentrations	[12]
Manganese nanoparticles	Improve photosynthesis, growth (max at 0.05 mg/mL), shoot length (10%), root length (2%), fresh biomass (8%) and dry	Manganese toxicity resulting in browning of leaves, extending to the whole interveinal area	[14]

	biomass (100%) in mung bean		
Boron nanoparticles	At 90 mg/L it increases seed yield, plant height, and number of pods Increase production of *Medicago sativa* under calcareous conditions	Boron toxicity causes necrotic lesions in leaves, resulting in premature senescence.	[12]
Molybdenum nanoparticles	Increase in yield and disease resistance in chickpeas	Molybdenum toxicity leads to the accumulation of anthocyanins in leaves, turning them purple in tomato and cauliflower, and yellow in legumes.	[12]
Nickel nanoparticles	Increase in chlorophyll a and b at 0.01 mg/L	Nickel toxicity leads to stunted growth, chlorosis, necrosis, and wilting.	[12]

TiO_2 nanoparticles	Enhances nitrogen fixation and photosynthesis in spinach	Variation in size of the nanoparticle results in some being able to penetrate the plant pores and larger particles get accumulated in the soil. Accumulation in the soil leads to disturbances in soil enzyme concentrations	[15]
Cerium oxide nanoparticles	Improvement in growth and nutritional values at lower concentrations At 100 mg/kg conc. it enhances the growth of *Lactuca sativa* Improved rate of photosynthesis in soybean in high moisture soil	High concentrations inhibit growth in *Lactuca sativa* Can't improve the photosynthesis rates in soybean due to low moisture soil	[12]
Silver nanoparticles	Promotes plant growth and seed germination Antimicrobial effects	Affects nitrification efficacy in soil Can affect root and shoot growth and also the germination in Mung bean at 3000-6000 µg/mL	[12]

Gold nanoparticles	Enhances the root to shoot length ratio in *Lactuca sativa* with no toxicity Improves germination and physiology in maize seeds	Translocation of the nanoparticle is difficult at lower concentrations in some plants, while it's observed in some others, suggesting a difference in the bioavailability.	[18], [12]
Nano-cobalt powder	Improves chlorophyll index, nodules, and yield in soybean	High concentrations can result in iron deficiency, loss of leaves, and paling of leaves	[12]
Selenium nanoparticles	Improve the production of amino acids, flavonoids, proteins, phenolic compounds, etc. Antimicrobial, antioxidant, and anti-cancer properties	Selenosis or selenium toxicity leads to higher oxidative stress and distortion of protein structure and function	[12]
Single-walled carbon nanotubes	High germination rates in crops	High levels result in toxicity, leading to necrosis and apoptosis	[15]

Multi-walled carbon nanotubes	Improves the uptake of nitrogen and proteins from wastewater	Could reduce chlorophyll content and cell viability	[15]
Nano-silicon dioxide	Enhances growth and resistance to both abiotic and biotic factors Enhances structural strength of plants Improve seed germination in *Lycopersicon esculentum* when treated with 8 g/L of 12 nm nano-silicon oxide	Affects plant height, shoot, and root biomass Can affect translocation of nutrients like copper, magnesium, and sodium	[12]
Clinoptiloloite zeolites	Binds with ammonia, and the zeolite bound ammonia acts as a great slow-release nitrogen source Enhances efficiency of associated fertilizers.	Reduces performance at low temperatures Coarsening of mean grain size in the presence of water	[14], [16]

The main focus is on generating cell-based adipose (i.e., fat) tissue for plant-based meat; given its significant contribution to taste and texture. Adipose tissue can be found in cell-based meat, an animal meat alternative cultured in-vitro in laboratories using animal cells that have extracellular heme proteins. This protein imparts sulfur to the "bloody" flavor of meat and also helps in improving the color of the meat. Whereas in plant-based meat heme proteins were absent [19]. If heme protein along with sulfur nanoparticles can be incorporated inside the plants through nano fertilizers it might bring flavor to the plant-based meat. Apart from textural properties, myriad factors namely impact of cooking, cell to scaffold ratio, packaging, storage, and shipping on tissue culture need to be considered. The texture is influenced by both supportive scaffolding materials and cultured cells. Differentiation and cell alignment strategies are used to mimic muscles. In in-vitro to induce cell alignment micro-patterned substrates, mechanical tension, and electrical stimulation can be applied [20, 21].

The amount of protein present in wheat, pea, and soy is at par with animal-based meat. However, it is important to ensure balance in protein complementation and amino acid profile. Legume and cereal proteins are favorable complements. Resistant in plant protein structure during proteolysis and its conformation and anti-nutrients (e.g., tannins, phytates, lectins) decrease nutrient bioavailability post-ingestion. Soaking, heating, and sprouting methods increase the digestibility of plant proteins. A different set of nutrients are obtained from different cell types and fatty acids from matured adipocytes. The differentiated muscle cells will likely be the primary source of protein.

Some nutrients that are present in animal-based meat are absent in cultured cells. For example, vitamin B12 is only synthesized by bacteria and would need to be supplemented with meat substitutes. Cell-based meat has a similar nutrition profile to animal-based meat and the slight discrepancies in the nutrients can be balanced by using media supplementation, co-cultures, and genetic modification [20]. Genetic engineering is another approach to improve the nutrients requirements and it had already been applied in livestock to improve meat production. However, consumer acceptance and regulatory strategy are the main problems of this approach [23]. Similarly, an improvement of the protein and amino acid profiles in the plant products will improve the nutritive factor in plant-based meat.

3. Previous studies showing improvement in plant nutrients using nano fertilizers

Recent studies shows that the nano fertilizers improves the plant nutrients and such nutrient-rich plant sources can be used to improve the quality of plant-based meat.

Pea protein isolates and rice protein isolates were used as meat extenders at 3%, 6%, 9%, and 12%. Compared to control, rice and pea protein-enriched nuggets had higher protein values, ranging from 32.84% to 39.31% and 39.23% to 48.49%, respectively [24]. These types of protein replacements can be used to improve the nutritional value of plant-based meats. When compared to the control samples, all the treatments showed a substantial decrease in cooking loss and an increase in water-holding ability.

Nano-KH_2PO_4 improves the rice quality by increasing the water use efficiency. Rice along with soya and pea serves as a major component of plant-based meat. Both soya and pea have an albumin and globulin fraction. Nano fertilizers raise albumin levels during seed germination and enhance salt tolerance in these plants, allowing for further globulin extraction. Thus, the protein levels in the soy and pea plants are increased. When such protein-rich plant sources were used it led to producing protein-rich plant-based meat [24].

At different pH levels, low and high concentrations of iron oxide and hybrid platinum decorated iron oxide nanoparticles were used as nano fertilizers to investigate their effect on embryonic root growth in legumes. At low concentrations, iron oxide nanoparticles were found to increase root growth by almost 80 percent using a combination of material characterization techniques and a statistical analysis process [25]. The legume crops like beans, chickpeas, and lentils are highly used as the protein source in plant-based meat. Thus using nano fertilizers would enhance the root growth as a result the uptake of nutrients from the soil will be increased leading to produce nutritive-rich legume crops [26].

Studies have found that ZnO nanoparticles have an important particle size, morphology, and concentration-dependent effect on seed yield, lipid peroxidation, and various antioxidant biomarkers in soybean [27]. It has been documented that nanoparticles and nanotubes have improved germination, seedling development, and physiological activities such as nitrogen metabolism, protein levels, photosynthetic activity, mRNA expression, and positive changes in gene expression in crops namely sunflower, common bean, and maize [28]. When such crops are used as nutritive-rich raw material they will improve the quality of the plant-based meat.

Enzymatic hydrolysis is a mechanism in which enzymes aid in the cleavage of bonds by using water as a catalyst. Nanoparticles applied using nano fertilizers into leguminous plants play a significant role in enzymatic hydrolysis which further helps in the development of protein structure, peptide profile induction, solubility enhancement, good flexibility of texture, better digestion, etc. [29]. Enzymatic hydrolysis of soy protein resulted in improved solubility, foaming, and emulsifying ability. In addition, it improves nutritional value by enhancing functional properties. Functional food additives, flavor, nutritional enhancers, protein substitutes, and clinical products are popular uses of soy protein hydrolysates [29].

It was discovered that foliar application of NPK nano fertilizers (Nitrogen-Phosphorus-Potassium equally balanced) at a 50% rate was the most cost-effective treatment, as it resulted in the high potato yield, quality, and cost ratio of potato production. To retain moisture in plant-based meat, potato protein is used as a source thus using the high-quality potatoes as mentioned above would enhance the quality of the meat [30].

Now, researchers at the Agarak Research Institute in New Delhi have demonstrated that using nano fertilizers at the right doses can improve the nutritional quality of wheat by increasing its zinc content. The majority of Indian soils are zinc deficient, so adding zinc to the soil along with NPK fertilizer is highly recommended. Wheat protein is added to plant meat to make it chewier. Treating wheat with zinc nano fertilizers enhances the wheat quality and it can be used as a source to produce a quality of plant-based meat [31]. Gluten which is also an important source of plant-based meat has unique cohesive and viscoelastic properties that can form fibrous proteinaceous networks commonly used in plant-based meat products [32]. Treating gluten with zinc nano fertilizers would do the same quality enhancement as in wheat.

Leguminous plants are another source of plant-based meat which contains heme, a key oxygen-binding component that aids in the taste and odor of plant meat. Using nano fertilizers improves the amino acid composition in leguminous plants. Crop breeding is primarily focused to increase micronutrients and vitamin A levels in crops [33]. If the crops are improved in terms of quality and protein (enriched with a high bioactive profile) then they can be used as a nutrient source for plant-based meat [30].

The soil requires nitrogen as a single nutrient to improve crop productivity. Anhydrous urea, ammonia, and urea–ammonium nitrates are commercially available in solid or liquid form [34]. It is sprayed in soil, where it reacts with water to release ammonium ions, which are then nitrified by bacteria into the nitrate ions that plants need. Farmers often apply excess fertilizers to ensure the best harvest, but such activities may be detrimental to the soil because they alter the structure and concentration of the soil. Phosphorus is an important component of plant nucleic acid structure and it is a major plant nutrient for protein synthesis. It plays a role in cell division, tissue growth, and complex energy transformations in plants. Energy is stored in phosphate compounds, which are generated by photosynthesis and carbohydrate metabolism [32]. All these nutrients can be supplied to the crops using nano fertilizers. Higher the quantity of nutritive compounds in crops, better the quality of plant-based meat.

Organic farming or bio fertilization produces high-value crops, but it is done on a small scale, particularly by rural farmers, and the product is expensive. Thus, nano fertilizers can revolutionize agricultural systems by producing high-quality crops, and using such crops as sources to produce plant-based meat will enhance the meat quality. Plant-based meat will attain a high peak in the forthcoming years and improving plant-based meat would result in the improvement of food habits [32].

4. Benefits and drawbacks of nano fertilizers

Nano fertilizers are produced with the aim of stable and sustained delivery of micro and macronutrients required for the growth and development of the plant. This can overcome the drawbacks of conventional fertilizers; especially the limited bioavailability and utilization of nutrients by the plant. The loss of nutrients in the soil can be significantly reduced using nano fertilizers because of their foliar application. Generally, nutrients like sulphur have to be oxidized by soil microbes before they are taken up by the plants. This process, dependent on the size of the particles and thus the uptake of such nutrients can be accelerated by using nanotechnology and foliar application [3].

Other than the advantages of using nano fertilizers they also have certain disadvantages. The nanoparticles may persist in the plant systems and soil ecology for a long time and continue the steady release of the nutrients which could lead to toxicity, destroying both the plants and the soil ecosystem [8]. As the nano fertilizer is in a growing phase there is no system in place to manage its risk and its production processes are costly. However, once the technology is refined it can be made available at cheaper costs than conventional fertilizers [9]. Nano fertilizers with the benefits and drawbacks in the plant and soil ecosystem are discussed in Table. 1.

5. Conclusion

A drastic increase in the world's population leads to demand for food products. As a result, there is a need for a high quantity of agricultural products. Nano fertilizers can be used as an alternative to conventional fertilizers due to their high cost and adverse side effects. They have the potential to improve overall plant-growth characteristics due to their smaller size, high surface area to volume ratio, improved chemical stability, enhanced reactivity, high ionization power, high pH tolerance, adequate absorbability, and thermal stability.

Animal meat products; especially, intake of red meat leads to health effects such as heart disease, cancer, and diabetes, etc. Nano fertilizers aid plant growth by improving nutritive factors, bioactive substances, and overall gene expression for the benefit of the plants, and they can be used as the main source for producing plant-based meat. A quality plant-based meat mimicking animal meat in terms of organoleptic properties and nutritional parameters leading the vegetarians to enjoy the taste of meat without killing the livestock. In near future, plant-based meat will attract a large population which leads to an increase in production and the establishment of new plant-based meat companies.

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