

## Development Of Nanobiofertilizer Via Chemical & Biological Synthesis

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**ABSTRACT:** The major challenge of modern agriculture is to satisfy actual and future global food demands efficiently. This great challenge requires combined efforts to preserve natural resources to support intensive agriculture while limiting detrimental impacts on the environment. One of these efforts is using nanofertiliser. Nanofertiliser is the application of nanotechnology in biological science. Nanotechnology is the science of manipulating materials at the nanoscale (1nm=10<sup>-9</sup>m). This review summarizes the potential of nanofertiliser for its importance in increasing yield in agriculture and providing consumers with quality and contamination-free food. In the agriculture sector, nanobiotechnology is necessarily used as fertilizers (nanofertilizers) for crop yield improvement, pesticides (nanopesticides) for crop protection, and nanobiosensors for the detection of crop pathogens, soil conditions, and vegetation conditions. Similarly, intelligent food packaging, and detection of pathogens, adulterants, and toxins in food are its importance in the food sector.

**Keywords:** Nanotechnology, Biofertilizer, Nanofertilizer, Nanobiosensors, Nanobiofertilizer

### INTRODUCTION

The current international population is exceeding about 7 billion, and these large proportions are living in developing countries and suffer from the limitation of food as a consequence of dynamic climate conditions and depleting resources. Agriculture is the backbone of development as it provides food for humans. To increase agricultural productivity, some technologies such as fertilizers and pesticides were widely utilized in Africa as well as in the world. However, the overuse of chemical compounds has reported serious issues such as hardening of the soil, decrease in soil fertility, air and water contamination that may kill soil friendly microorganisms, and destabilization of the ecosystems. For example, over 500 species of pests have evolved a resistance to a pesticide, analogous to the bacterial antibiotic resistance; it causes farmer to use stronger pesticides in more quantity, some of which are quite toxic. This great challenge will require combined efforts to support intensive agriculture while limiting adverse effects on the environment. One of these efforts is using nanobiofertilizer that has been known as a possible technology with new tools for increasing yield in agriculture and providing high-quality food products for humans.

Nanobiofertiliser is taken into account to be the distinctive fusion of nanotechnology and biology. Nanotechnology could be a scientific technique that manipulates a substance at molecular levels to nanoscale up to 100 nm particle size. A nanometer (nm) equals one-billionth of a meter. On the other hand, the study of any sub cellular entity will be thought-about as nanobiology. The typical protein, hemoglobin has a diameter of about 5nm, and DNA double helix that has 2nm wide is comparable in size to nanomaterials. Nanobiofertiliser is the use of living organisms or their components to produce useful products or processes for a particular purpose. Nanobiofertiliser is fertiliser at the "nanoscale" or application of the tools and processes to study and manipulate biological systems. According to Fakruddin, nanobiofertiliser is the incorporation of nanomolecules into the biological system to benefit life. Similarly, Rao defines it as a use of the biological methods of engineering, construction, and manipulating entities in the range of 1 to 100nm. The association of those two technologies has the potential to enhance the production of useful products in the agri-food system and also develop our current concepts and understanding.

In the agriculture sector, nanobiofertiliser is possibly necessary to improve crops, enhance the production of secondary metabolites, be used as fertilizers and pesticides, detect crop pathogens fast and early, and monitor soil conditions. As mentioned by Shaimaa et al., agricultural land and soil pollution present in industrial and wastewater are the most important factors limiting crop and food production. Thus, nanostructure catalysts will detect and eliminate the harmful element of agricultural ecosystems as a safe.

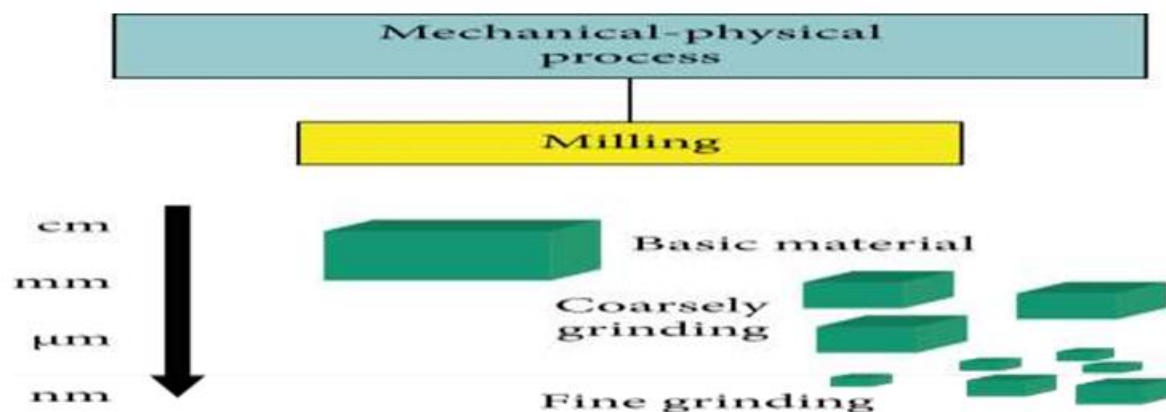
The production of quality and safe food based on consumer needs is another area that requires great attention. To make sure food quality and safety, procedures such as high sensitivity and low detection limit, for easy sample preparation steps, are widely utilized at the laboratory level. However, these methods need time ranging from many hours to a day. Nanobiofertiliser - based nanosensors/nanobiosensors may detect pathogenic bacteria, adulterants, and toxins in food within few minutes to an hour. Although the contribution of nanobiotechnology such as diagnostic, therapy, and drug delivery has deep-rooted in the

medical sector, the role of nanobiofertiliser within agri-food systems remains at its infancy stage. Some studies were performed on the role of nanobiofertiliser within the agri-food system showing some aspects of this technology. Therefore, this paper aimed to review studies made by various scholars on the importance of nanobiofertiliser within the agri-food system.

## METHODS OF NANOPARTICLE PRODUCTION

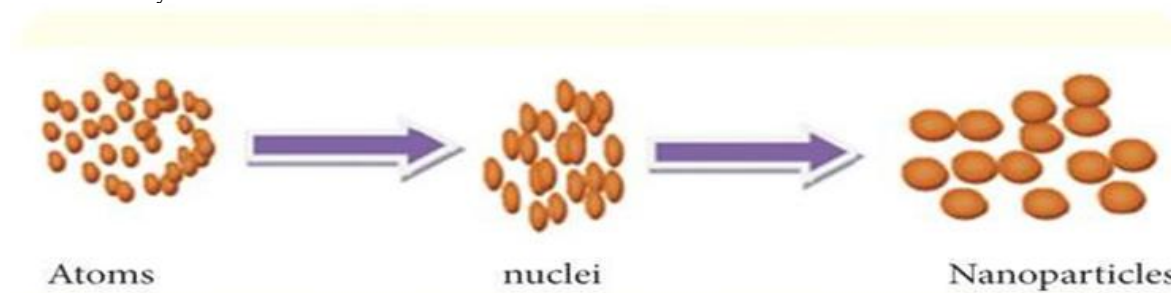
### Top-Down Approach

The top-down approach involves the process of breaking down bulk substances into nanoscale particles. In this approach, the imperfection of surface structure may be the problem. The device typically involves optical and electron beam lithography.



### Bottom-Up Approach

The bottom-up approach involves the build-up materials for different purposes. For this, physical, chemical, and biological approaches that cover the following fields, such as material science, chemical engineering, biochemistry, and molecular biology, are used. Physical and chemical methods are costly and could also have toxicity. But biological methods are cheap and eco-friendly.



### Biological Mediated Nanoparticle Synthesis

Within the biological approach, several living organisms such as bacteria, yeasts, fungi, plants, algae, and marine organisms were reported as important agents that synthesize NPs with different shapes. As suggested by Ghosh et al., NP production is a way of stress response and biodefense mechanism for the microbe, which involves metal excretion/accumulation across membranes, enzymatic action, and precipitation. The study by Barabadi et al. reported that nanoparticles are biosynthesized by enzymes generated by cellular activities of the microorganisms after they grasp ions from their environment. Thus, nanoparticle synthesis is categorized into intracellular and extracellular based on the location where they are formed. The intracellular technique consists of transporting ions into the microbial cell to make NPs within the presence of enzymes. Explained the intracellular synthesis of gold NP by fungus *Verticillium* sp. and silver (Ag) NP by bacterium *Pseudomonas stutzeri* AG259. The authors put isolated silver in a concentrated solution of silver nitrate, which showed a reduction in the Ag<sup>+</sup> ions and consequently led to the formation of silver nanoparticles (AgNPs). But, the precise mechanism for the intracellular formation of gold nanoparticles by *Verticillium* sp. was not fully understood. Magnetotactic bacteria are another example of bacteria that synthesized iron oxide and iron sulfide intracellularly that are known to be biocompatible.

The technique of extracellular synthesis of NPs includes trapping the metal ions on the surface of the cells and reducing it within the presence of enzymes. Win et al. explained fungus-mediated AgNPs synthesis. conjointly explained in their report that the fungus, *Fusarium oxysporum* synthesizes gold nanoparticles extracellularly. The gold was first trapped on the surface of the cells via interaction between the ions and negatively charged cell walls from the carboxylate groups in the enzymes. Then, the enzymes reduced the metal ions to create gold nuclei and then grow through more reduction and accumulation.

Plants also have the potential to be exploited to prepare NPs. Leaves of a geranium plant (*Pelargonium graveolens*) have been used to synthesize gold nanoparticles. Biomolecules, such as flavonoids, phenols, protein, and others, have an important role in the reduction of metal ions and the topping of gold nanoparticles in plant extracts represented the synthesis

of copper oxide nanoparticles (CuO NPs) using plant leaf extract of *Catha edulis*. According to his findings, the biosynthesized CuONPs were extremely crystalline and were found to be pure. Some other nanoparticles produced by microorganisms and plants are listed.

TABLE 1: Some of the nanoparticles synthesized by microorganisms/plants and their possible role in the agri-food system.

Microbial strain	Plant/part of plants	Nanoparticle	Use of nanoparticle	Reference
<i>Lactobacillus</i> sp.		TiO <sub>2</sub>	Antibacterial activity	[11]
<i>Thermomonospora</i> sp.		Au		[11]
<i>Pseudomonas stutzeri</i> AG259		Ag	Antimicrobial activity	[12]
<i>Fusarium oxysporum</i>		Au		[13]
<i>Corynebacterium glutamicum</i>		Ag		[14]
<i>Pseudomonas aeruginosa</i>		Au	Antimicrobial activity	[14]
<i>Aspergillus flavus</i>		TiO <sub>2</sub>	Production of secondary metabolites	[15]
<i>Staphylococcus aureus</i>		Ag	Antimicrobial activity	[15]
Magnetotactic bacteria		Fe <sub>2</sub> O <sub>2</sub>	Isolation of DNA of milk pathogenic bacteria	[15]
<i>Candida utilis</i>		Au		[16]
<i>B. subtilis</i>	Leaf extract of <i>Catha edulis</i>	Cu		[17]
		Au	Dye degradation	[18]
	Leaves of <i>Pelargonium graveolens</i>	Au		[18]
	Aloe vera	Cu		[18]
	Leaves of <i>Catharanthus roseus</i>	Ag	Enlargement of plant height	[18]
	<i>Medicago sativa</i>	Ag	Enhancement of germination	[18]
<i>Escherichia coli</i>		Au		[19]
<i>Rhodococcus</i> sp.		Au		[19]
<i>Verticillium</i> sp.		Au		[20]

**TABLE.....**  
**Nanobiofertiliser in the Agriculture Sector**  
**Nanopesticides**

Agrochemical technology aims to protect the crop against plant pathogens, weeds, and harmful insects that reduce agricultural yield. Today, the utilization of pesticides is the quickest and the most cost-effective way to control pests and diseases. But uncontrolled use of pesticides has caused several adverse effects on human health, domestic animals, pollinating insects, and ecosystems in large, with rising demand for new technology. The use of nanopesticide formulation could provide better opportunities compared to chemical pesticides. Nanopesticide refers to pesticide formulated in nanomaterials for plant disease protection. The nanopesticide formulations can increase water solubility and bioavailability and protect agrochemicals against environmental degradation, revolutionizing the control of pathogens, weeds, and insects in the crops. However, indiscriminate use of nanopesticides may affect water quality, and human health due to their high ability to the distribution and bioaccumulation in soil, water, and food. The use of nano encapsulation, an environmentally friendly method for crop protection, could be an appropriate solution for this drawback. Nano encapsulation is a technique of surrounding biologically active ingredients into capsule walls that can be released to the environment under controlled conditions when external stimuli activate the capsule walls to break, melt, or dissolve slowly. Nano structured matrix systems such as nanocapsules, nanospheres, and nanovesicles could be designed and utilized using sensitive polymer materials to deliver pesticides. A recent study by Bhabesh explained that nanoparticles as nanocarriers are used as a pesticide delivery system to release active ingredients to a specific target site at a specified time duration by minimizing the harmful effects on non-target organisms. The main thing in the utilization of nano pesticides in agriculture could be the restriction of accessible strategies used for pest control.

Shaimaa studied the effect of Ag and Ag-Zn NPS as pesticides with different concentrations on *Aphis nerii*. The result showed that Ag NPS is used as a valuable tool in pest control programs of *A. nerii*. To boot, the study showed that imidacloprid at 1µL/mL and NPs at 700mg/mL had the very best insect mortality effect. Nanosulfur formulation (AR Inano-S, 50–90nm) successfully inhibited the conidial germination of *Erysiphe cichoracearum* (powdery mildew fungi) at 1,000ppm and was found superior to commercial sulfur product. John et al. reported the potential of zinc oxide nanoparticles (ZnONP) and CuNP in controlling plant pathogenic fungi and bacteria at low doses. According to his finding, the bacterial blight of pomegranate (*Punica granatum*) caused by *Xanthomonas axonopodis* pv. *axonopodis* was treated by CuNPs (at the concentration of 0.02ppm) and found to suppress *X. axonopodis* pv. *axonopodis* growth. The larvae of case-bearing cloth moths, *Tinea pellionella*, were successfully killed when treated with ethanol-based nanosilver colloid (<20 ppm).

**Nanofertilizers**

Substituting nanofertilizers for conventional approaches to fertilizer utilization is a mechanism to release nutrients into the soil in a controlled way, thus preventing nitrification and pollution of water resources. Nano fertilizers have distinctive options such as production enhancement, rise in photosynthesis, and significant expansion in the leaves' surface area. The issue that needs consideration in nano fertilizer utilization may be because of their smaller size they can easily escape into the atmosphere and cause environmental problems. Thus, in nanofertilizers, nutrients should be coated with nonmaterials and delivered as nanoparticles under controlled conditions. This technique of nanoparticles utilization can mitigate the different environmental issues and increase the supply of nutrients within the rhizosphere. John et al. Showed that the application of a nanocomposite consisting of N, P, K, mannose, and amino acids increased the uptake and use of nutrients by grain crops.

Gholamreza explained that treatment with tin dioxide NP (TiO<sub>2</sub>NP) on maize had a substantial result on growth and alleviated salinity stress effects on Moldavian balm (*Dracocephalum moldavica* L.). According to this study, the application of 100 mg/L TiO<sub>2</sub>NP on Moldavian balm grown up under stress salinity level (100 mM NaCl) improved agronomic yield compared with plants grown under salinity without TiO<sub>2</sub>NP treatment. Thus, TiO<sub>2</sub> enhanced light absorption and energy transmission. In another research, silver dioxide (SiO<sub>2</sub>) and TiO<sub>2</sub> NPs increased the activity of nitrate reductase enzyme in soybeans and intensified plant absorption capability, making its use of water and fertilizer more efficient. Pasupuleti conjointly verified that nanosized TiO<sub>2</sub> and SiO<sub>2</sub>NP together could enhance growth and water and fertilizer use efficiency and accelerate germination in spinach (*Spinacia oleracea*), wheat (*Triticum aestivum*), tomato (*Solanum lycopersicum*), and maize (*Zea mays*). Marjan et al. also reported that the application of 15 mg/kg of nano-Fe/SiO<sub>2</sub> increased the shoot length by 8.25% and 20.8% for barley and maize, respectively. During a study by Mohd War et al. the effect of soil-applied Ag NP at concentrations of 25–50 ppm on wheat plants showed enlarged plant height and fresh and dry weights as compared to control. Thus, sensible use of Ag NP in soil can improve the yield of wheat; however, further investigations need to be taken into consideration to realize the potential of Ag NP in growth and yield improvement in alternative crops.

Currently, many secondary metabolites are potential sources of nutraceuticals, pharmaceuticals, and agrochemicals. However, those secondary metabolites are produced in very low quantities by plants. To increase the production of secondary metabolites, NPs play a vital role due to their novel and unique properties as reported by Mohd. According to his report, TiO<sub>2</sub>NPs enhanced the production of secondary metabolites such as flavonoids, and phenols by cell suspension cultures of aloe vera.

### **Nanobiosensors**

The current advancement in science and technology-enabled farmers to easily detect plant pathogens (diseases), insecticides, herbicides, pesticides, and fertilizers and to monitor soil conditions with help of precision farming. Precision farming is the use of computers system, satellites, drones, and the internet of nanotechnology (IoT) to measure environmental conditions such as nutritional deficiencies, irrigation problems, and parasitic attacks using nanosensors, thereby figuring out whether crops are growing at maximum efficiency or not and livestock management.

Nanosensors in agriculture and food analysis use the aggregate of biotechnology and nanotechnology and may also be called nanobiosensors. Nanobiosensor is nanostructured devices that have physical-chemical transducer and biologically derived sensitized agent. The components are transducer, detector, and biological recognition elements. The biologically sensitized element can be cells, tissues, enzymes, receptors, antibodies, nucleic acid, and aptamers, which are utilized for the production of a signal. Mukhapadhyay explained in his finding that nanobiosensors work with the aid of triggering an enzymatic reaction through the use of nanoengineered branching molecules referred to as dendrites as probes to bind to target chemical substances and proteins. Identification and utilization of biomarkers that accurately detect the exact stage of the disease is also a new area of research.

#### **1) As a Device to Diagnose Disease.**

The problem with disease control lies with the detection of the precise stage of prevention. Most of the time application of pesticides after the appearance of the symptom leads to some amount of crop losses. Additionally, currently, some diseases, notably viral diseases, are difficult to control once it starts showing their symptoms. Thus, nano biosensors help facilitate in successful control of disease by detecting the precise stage of viral DNA replication. For instance, nano-based viral diagnostics, together with multiplexed diagnostic kit development, have taken momentum to detect the strain of the virus and the exact stage of treatment of some therapeutic to prevent the disease.

Agricultural drones for crop health assessment are interesting technology that uses nanobiosensors. In this case, sensors may indicate their health at the early stage and calculate their vegetation index. Multispectral sensors allow field areas affected by the infection. Based on data, the exact amount of chemicals needed to eradicate this infestation.

#### **(2) As a Tool to Detect Pesticide, Soil Conditions, and Livestock Management.**

Many nanobiosensors are designed to detect pesticide and soil conditions and livestock management. For pesticide detection in soil, the conventional method, the chromatographic one, is inefficient but complex and cost. For this, using nanobiosensor as an alternative to detect pesticides could be the preferable method. Liposome biosensors can detect organophosphorus pesticides at very low concentrations such as dichlorvos and paraoxon.

The soil conditions such as pH, temperature, moisture content, nitrogen, potassium, and phosphorus contents can be monitored using nanobiosensors. Thus, the internet of nanotechnology (IoT) is connected to users and other devices via the Internet, increasing the quality and sustainability of agricultural products. It has a sensor, particularly nanobiosensors, that can collect information about livestock management and soil conditions such as pH, moisture content, and nutrient content and detects real-time weather conditions. External devices can then integrate the data and automatically generate a report about potentially devastating changes in their environment. Afriscout cell phone applications to detect surface water and vegetation conditions practiced by pastoralist communities in Ethiopia, Kenya, and Tanzania are examples of IoT.

Agricultural drones for field analysis are another interesting technology. In this situation, drones with multispectral sensors can be used to collect information. The sensors may be placed inside or outside of the agricultural fields and detect real-time weather conditions such as humidity, temperature, and rainfall. These can assist farmers to utilize inputs more efficiently, thus developing sustainable agriculture.

## NANO BIOFERTILIZER IN THE FOOD SECTOR

Nanobiofertilizer has potential applications in all of the processing, packaging, and transport of food products. Food processing is the method used to convert raw materials into edible products making them palatable with long shelf life. The process involves chopping, removing toxins and pathogens, and adding an ingredient to increase shelf life and packaging. Thus, processed foods are generally less susceptible to early spoilage than fresh foods and are suitable for long-distance transportation. Today, most food quality and safety analyses performed by conventional laboratory methods can test only a small number of samples. Detection of food contaminants and adulterants at a low level using conventional laboratory methods is a difficult task. Thus, utilizing fast and high sensitivity techniques could be a method of choice for food makers, storage facilities, and consumers to keep food quality and well-being throughout processing, packaging, and transportation.

Nanobiofertilizer is one of the platforms that can monitor food quality and safety. These platforms depend on either chemical transduction systems or biomolecular recognition for increased selectivity. The biosensors contain biological receptors such as enzymes, aptamers, cells, and antibodies that can explicitly perceive a target, and the

binding process is done by electrochemical and optical processes. The biosensors distinguish changes in cells/molecules that are then used to recognize the test substance. At the point when the substance ties with the organic part, the transducer delivers a sign relative to the amount of the substance. In this way, in case, there is a huge convergence of microscopic organisms in a particular food; the nanobiosensor will deliver a strong signal demonstrating that the food is unsafe to eat. Exploration by various researchers revealed that nanobiosensors can distinguish microbes such as *Escherichia coli*, *Salmonella*, poisons and heavy metals, nutrients (antioxidant and sugars), and adulterants with the possibility to overcome the constraints of exorbitant and laborious laboratory techniques. Nanomaterials ordinarily utilized for food quality and safety analysis are Au NPs, Ag NPs, attractive iron dioxide NPs ( $\text{Fe}_2\text{O}_2$ NPs),  $\text{TiO}_2$ NPs, Zn, and  $\text{ZnO}_2$ NPs.

### Pathogens and Toxins Detection

The presence of pathogenic organisms in food is a serious issue that needs special attention. The failure in the detection method may result in food spoilage and complicated health issues. The food-borne pathogenic organisms such as *Listeria monocytogenes*, *E. coli*, *Staphylococcus aureus*, *Bacillus cereus*, and *Salmonella* spp. were major causative agents of food-borne illness. The common DNA detection method, PCR, is more expensive, and the 16s technique lacks sensitivity. The current advancement in nanobiotechnology provided a technique to detect pathogenic organisms that contaminate food. Nanoparticle-assisted deoxyribonucleic acid (DNA) detection and microorganisms' identification were less tedious and more delicate than other techniques. In this case,  $\text{Fe}_2\text{O}_2$ NPs have been utilized for isolating DNA of *L. monocytogenes*, milk pathogenic bacterium. The  $\text{Fe}_2\text{O}_2$ -NPs-based detection of 16s ribosomal ribonucleic acid is simple and more dependent on a sandwich hybridization technique, wherein two oligonucleotide probe binds to each end of the target nucleic acid. Complete RNA is extracted from a specimen, and the target region inside the 16s rRNA is amplified by unbalanced opposite record PCR (RT-PCR) to deliver enormous quantities of single-strand DNA with just sense (or antisense) sequences. The resultant DNAs are then caught by polymeric microspheres conjugated with test oligonucleotides (the bead capture probe). In this manner, the overhanging edges of the objective DNA are hybridized with MNP-detection probe conjugates. In other studies, by Worku et al., *E. coli* and *Salmonella* spp. have likewise been distinguished utilizing an Au NPs surface plasma resonance (SPR) measure created for use in a crude food sample. The sensor was created by modifying an SPR chip with poly(carboxy betaine acrylamide) (pCBAA) brushes with a 20 nm thickness, followed by binding of antibody onto their surface. The concentrate containing microscopic organisms was deposited on a surface, and then secondary biotinylated Ab and streptavidin-coated spherical Au NPs were added. The method was able to detect down  $17 \times 10^1$  CFU/mL for *E. coli* and  $11.7 \times 10^3$  CFU/mL for *Salmonella* in extracts within 80 minutes.

### Adulterant Detection

The presence of toxins such as pesticides and heavy metals in foods causes diseases to humans. Consequences are also aggravated by the leaching of materials from materials used in packaging, unintended exposure. Bisphenol A (BPA), a product used in the manufacture of polycarbonate and epoxy resin, is a typical example. BPA is important in the production of different food packaging instruments such as water bottles, feeding bottles, and coating material for processed food cans. It is electrochemically active and can be identified by direct oxidation on the surface of the electrode that can be amended with metal NPs to improve detection.

Among other chemical hazards in food, mycotoxins pose particular challenges due to their toxicity at low exposure levels. Nowadays, there is no suitable method for detecting mycotoxins, which makes it difficult to identify. Even small concentrations of mycotoxins can cause significant health effects, such as vomiting, kidney, liver, cancer disease, and death. There is a need for a sensitive and reliable methods for their detection. The wide application and reliability of nanobiosensors make it a method of choice. Ochratoxin A (OTA) is a nephrotoxic toxin, induced by *Aspergillus ochraceus* and *Penicillium verrucosum*, with strong carcinogenic effects on rodents, as well as immunotoxin problems in humans. The nanobiosensor can functionalize the  $\text{CeO}_2$  particles with OTA-specific ssDNA aptamers forming in higher dispersibility and activity. Changes in redox properties at the  $\text{CeO}_2$  surface upon binding of the ssDNA are measured using TMB, enabling rapid detection of OTA.

Adulterants such as food dyes are added to modify the properties and appearance of food. Sudan I is the most widely used food dye for food coloring. This dye has a carcinogenic effect on human health. Gold-nanorod-functionalized graphene oxide (GNRs/GO) has also been utilized to change the glassy carbon electrode (GCE) surface and used for the immobilization of Sudan I antibodies.



The technique of detection was depending on a competitive immunoassay where Sudan I was used to the modified electrode surface, followed by the addition of an antibody probe labeled QDs-PAMAM-Pd/Au CSNs.

Melamine, another common adulterant used in milk, is added to provide an apparent enhancement in protein content. In 2008, more than 290,000 infants were affected in China due to the adulteration of melamine milk. The incident raised significant safety concerns and the need to detect melamine in milk. Now, a localized surface plasmon resonance (LSPR) sensor chip is used to detect melamine in milk products using UV-Vis's spectrophotometry. To prepare the plasmonic chip, AuNPs were immobilized on a glass substrate with the chemical receptor, p-nitroaniline (p-NA). The sensor indicates high sensitivity to melamine when tested in samples of commercial milk powder.

## CONCLUSION

Many different opportunities for nanobiofertilizer exist to play an important role in the agri-food system. The ability of nanotechnology to chemical engineer matter at the smallest scale is revolutionizing areas. Productivity enhancement through nanobiofertilizer-driven precision farming and the provision of quality and safe food for a consumer is desirable. Nanobiofertilizer plays a substantial role as nanopesticide, nanofertilizers, biofertilizer and nanobiosensors in the agriculture sector. Nanobiofertilizer for detecting plant pathogens and determining real-time weather conditions such as humidity, temperature, rainfall, and soil conditions help farmers use their input properly. In the food sector, it has pathogen, toxin, and adulterant detection roles.

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