Bio-nanosensors: Synthesis and Their Substantial Role in Agriculture

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Abstract

Nanotechnology is a recent emerging area having vast potential in almost every field of science due to their small size and larger surface area as compared to bulk phase materials. Synthesis of nanoparticles can be done from physical and chemical methods, but these days, bio-nanotechnology is in demand that associate principles of biology with physical and chemical methods to synthesize nanomaterials having precise functions. In bio-nanotechnology, the nanoparticles are synthesized from biological means such as plants or microbes also called as plant-microbe-engineered nanoparticles (PM-ENPs). PM-ENPs are more efficient, less toxic and cost effective as compared to physical and chemically synthesized nanoparticles. Plant-microbe-engineered nanoparticles have good anti-microbial activity because of electrostatic interaction with cell membrane of microorganisms and electrostatic interaction build-up inside the cell cytoplasm. The PM-ENPs such as zinc oxide (ZnO) and sliver (Ag) are helpful in increasing the growth of plant by guaranteeing that the nutrients are used in controlled manners by the plants. Plant-microbe-engineered nanoparticles as bio-nanosensors have confirmed their possibility of success in agriculture. Bio-nanosensors can be used for monitoring of crop health, pests attack, environmental stressors and plant diseases. The bio-nanosensors can be used in pathogen detection, sensing food eminence, adulterants, dye, vitamins, fertilizers, taste, smell and pesticides. Therefore, plant-microbe-engineered nanoparticles have significant role in advancement of agriculture. This chapter will pave the path for the possibility of synthesis of nanomaterials by biological means such as by different plant parts and microbes. Also, the role of different metal nanoparticles in making of different types of bio-nanosensors and their substantial role in agriculture advancement have also been emphasized.

Keywords

Agriculture • Bio-nanosensors • Nanofertilizers • Plant-microbe-engineered nanoparticles

1 Introduction

Nanotechnology is coming into various fields such as biotechnology, engineering, food technology, agriculture and medical sciences and brought extensive research. It has an impact on all the forms of life because of its enormous use in automobiles, bio-medical sensors, catalyst, electronics, nano-fabrics, packaging, agriculture, bio-engineering, medicines, drug delivery, etc. (Shankar et al. 2004; Song and Kim 2009; Iravani et al. 2011). Nanotechnology is a novel discovery in the field of nanotechnology and changing too fast to cover thoroughly. Richard Feynman an American physicist in 1959 brought the concept of nanotechnology in a conference of the American Physical Society, where he gave the idea of the very vast potential of nanomaterials (Feynman 1960). When bulk materials are engineered into one or two dimensions in nano-range or smaller particles have properties which vary from those of the bulk phase material. Such engineered particles show totally different characteristics from bulk phase materials. The fact on which nanotechnology lies depicts that the reduction of size of the substances in nanometre range changes the properties of substances dramatically (Chattopadhyay and Patel 2016; Ail et al. 2017).

Bulk phase material is reduced to small size nanoparticles through different approaches such as top to bottom and bottom to up (Fig. 1). In top-to-bottom approach, nanoparticles are synthesized by physical and chemical methods which

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P. Singh et al. (eds.), *Plant-Microbes-Engineered Nano-particles (PM-ENPs) Nexus in Agro-Ecosystems*,
Advances in Science, Technology & Innovation,
https://doi.org/10.1007/978-3-030-66956-0_11

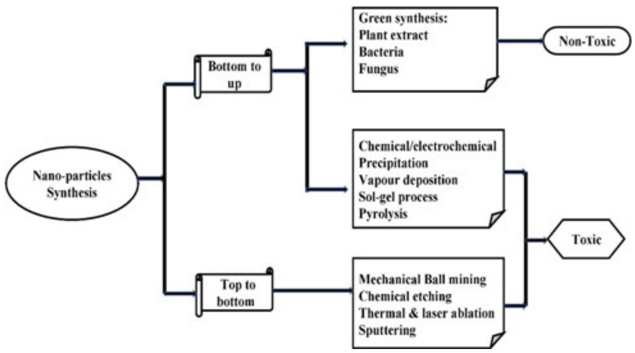

Fig. 1 Different approaches for the synthesis of metallic nanoparticles

mainly include grinding, cutting and etching, while bottom-to-up approach is self-re-arrangement of atom by atom or molecule by molecule for the synthesis of nanoparticles. There are advantages of using these approaches like possibilities to develop nanostructure with more homogenous chemical compositions and less defects. Nanomaterials are based on Gibb's free energy that is why such engineered nanoparticles are in thermodynamic equilibrium state or closure to this state. The top-down approach uses old methods to develop engineered nanoscale materials. Nano-scaled materials have different sizes, in combination with their different behaviour as well as have significant impact on chemical, physical, biological, electrical, mechanical and functional properties (Mukhopadhyay 2014).

This chapter will enhance our knowledge about the synthesis of plant-microbe-engineered nanoparticles (PM-ENPs) and how we can use these nanoparticles for development of particular bio-nanosensors. Besides this, the chapter will give a glance about the newest developments, applications of several nano-biosensors utilized in agriculture and relieving stress of minor population of farmers.

1.1 Synthesis Methods of Plant-Microbe-Engineered Nanoparticles

There are different methods of fabrication or synthesis of nanomaterials such as chemical, physical and biological methods. Methods such as physical and chemical have some disadvantages like high energy use, toxic chemicals and high cost. Therefore, synthesis by biological means has been evolved by use of animal-derived biomaterials, biomolecules of microbial origin and extracts of plant parts. Nanoparticle synthesis through plant extract and microbial means is called as plant-microbe-engineered nanoparticles (PM-ENPs).

Plants have been used as the main natural source for drug preparation and treatment of human illnesses. They are blessed by nature with a magical phenomenon to secrete secondary metabolites which are bioactive. These days, many natural products are made and used by the humans for treatments of various illnesses. Biosynthesis of nanomaterials is one of the current medicine manufacturing processes from the medicinal plant parts or from non-medicinal too. Nanoparticles possess exceptional properties due to their nanoscale size, morphology and distribution. Biosynthesis of nano-size particles from plant leaves, bark and fruit extract is cheap, environment-friendly and commercialized for large-scale production. There is least requirement of toxic chemicals, temperature and energy (Kharat et al. 2017). Biosynthesis of nanoparticles has become a subject of interest because of choices of reagents which are eco-friendly, whereas chemical synthesis method requires use of harsh chemicals for reduction and stabilization which makes it very expensive and harmful for agriculture (Sabir et al. 2014).

As synthesis process of nanoparticles by microbial cells is reliable, non-toxic and eco-friendly, biological organisms such as bacteria, viruses, algae, yeast and fungi have been used for synthesis of metal nanoparticles. Extracellular synthesis involves enzymes, proteins and organic molecules. Large number of enzymes, e.g. nicotinamide adenine dinucleotide hydrogenase (NADH) dependent reductase, naphthoquinone, anthraquinones and electron shuttle system, is machinery for reduction of toxic metallic ions into non-toxic metal nanoparticles (Patra et al. 2014; Bose and Chatterjee 2016). The mechanisms behind the extracellular and intracellular synthesis of nanomaterials are different among different microorganisms (Mandal et al. 2005; Hulkoti and Taranath 2014). In the intracellular synthesis, the positively charged metal ions are transported through the cell wall and interacted with negatively charged ions of the cell wall. However, in case of fungi, nanoparticle synthesis is extracellularly mediated by nitrate reductase in the presence of enzyme nitrate reductase helping in reduction of metal ions into nano-sized particles (Hulkoti and Taranath 2014).

1.2 Plant-Microbe-Engineered Nanoparticles Based Bio-nanosensors

Biosensors are devices which use biological or living entities for conversion of biological signal into electrical waves for general analysis, and the processor helps in quantification of signal. Biosensors have three functional units named as interactive sensor used for recognition, transducer for signal transfer and the processor which processes the signal transferred from the transducer. There are different types of biosensors such as immunosensors that uses antibody–antigen (Ab–Ag) reactions as a recognition model acting as binary mode. Other types of immunosensors are analytical immunosensor using Ag–Ab as the recognition molecule, in which Ab acts as a recognition entity for an antigen molecule and forms a stable reaction. There are a large number of applications of novel biosensors in research and development, food safety inspection agencies, food industry, food producers and policymakers who take an account for security and food safety (Prasad et al. 2017). However, there are some disadvantages while using traditional methods for detection of food quality such as expensiveness, time consumption which requires multiple steps for sample preparation before food quality detection and requirement of skilled technician and complex instruments which are not accessible to peoples of rural areas (Koedrith et al. 2014). The field of nanotechnology possesses capability to have strong impact in multiple fields such as energy, water, health, agriculture and food, and this is very profound technology among the new technologies.

Nanotechnology is the change of dimensions of bulk phase materials, system and devices at atomic and molecular level, in 1–100 nm range for developing new characteristics (Otles and Yalcin 2012; Prasad et al. 2015). Utilization of nanomaterials in construction of biosensors helps in overcoming the problems associated with old methods. There are advantages associated with nanosensors in place of old methods: they are highly sensitive and highly specific, offer accurate and rapid detection, and they are eco-friendly as well. Bio-nanosensor technology has the potential to detect analytes in low amounts (e.g. chemical or biological materials) that are dangerous to animals, humans and plants at a very low concentration, with very less preparation of sample and handy instrumentation. In field of agriculture, nano-based biosensors can offer opportunities for pesticide detection, drug residues, food-borne pathogens, heavy metal ions and toxic contaminants in foods in a very less time span. Also, nanosensors monitor crop stress, antibiotic resistance, soil conditioning, growth in plants, food quality and nutrient contents (Teodoro et al. 2010; Tarafdar et al. 2013; Prasad 2014; Prasad et al. 2014, 2015, 2017).

2 Types and Roles of Bio-nanosensors

Narayanan and Sakthivel (2010) has documented the large number of nanoparticles such as silver, cadmium, gold, magnetite, silica, titania dioxide, selenium, gold–silver alloy, copper, cobalt and platinum nanoparticles for formation of different types of nanosensors. All the metal nanoparticles and noble metals used are resistant due to corrosion, and hence, they are used for development of different types of utility nanosensors named as acoustic wave biosensors, magnetic biosensor, electrochemical biosensors, nanotube-based sensors, nanowire-based sensors. The functionality of every nanosensors is different.

- **Acoustic nanosensor**: It is used for amplification of the sensing responses and improving the preciseness of the detection limit.
- **Magnetic nanosensor**: It uses ferrite materials with transition metals. Electroactive species are monitored by electrochemical biosensors that are consumed or produced with activity of biological components.
- **Electrochemical nanosensor**: They are divided into potentiometric biosensors and amperometric biosensors. Potentiometric biosensors are not used frequently for checking food quality when compared with amperometric nanosensors having potential to monitor wide range of target analytes. Potentiometric nanosensors has been reported to detect monophenolase activity in apple juice (Dutta et al. 2001), and sucrose concentration detected in drinks (Rotariu et al. 2002), measuring fruit juices for isocitrate concentration (Kim and Kim 2003) and determines urea levels in milk (Verma and Singh 2003).
- **Calorimetric nanosensor**: It gives results of biochemical reactions in the form of heat absorption or production. Calorimetric transduction sensors detect heat consumed or generated in a biological reaction by using heat detection devices. They are used in detection of food quality and metabolites produced.

3 Biosynthesis of Bio-nanosensors Using Metal Nanoparticles

Various categories of metal nanoparticles are involved in the formation of metal oxide nanoparticles including magnetic and nonmagnetic, metal sulphide alloy, gold and silver nanoparticles. All these nanostructures can exist in diverse shapes such as nanoparticles, nanosheet, nanocomposites, nanotubes, nanorods, nanoconjugates, nanowires, etc.

4 Forms of Nanomaterials as Nanosensors

As nanosensors have high sensitivity and quick response, different types of nanomaterials allow quick penetration of fertilizers and nutrient for plant growth promotion, also act as nanosensors for quick monitoring of crop status and hence used in agricultural field such as pesticide detection, pathogen detection, insecticide detection, monitoring the crop biotic and abiotic stress and regulating plant growth. Predominantly, graphene oxide, multiwalled carbon nanotubes, multiwalled chitosan nanocomposite and ZnO chitosan nanocomposite are used enormously in all the mentioned application.

In pesticide detection, carbon nanotubes, gold nanoparticles, and nanocomposites and quantum dots with different polymers have been used (Cesarino et al. 2012; Liu et al. 2012). ZnO chitosan nanocomposite membrane was used for the detection of *Trichoderma harzianum* (Raskar and Laware 2014). Graphene oxides further detect content of nitrate in soil (Pan et al. 2016). Further, carbon nanotubes having single wall (SWNTs) when inserted into chloroplast of plant cells increased the photoabsorption (Wong et al. 2016). Kwak et al. (2017) demonstrated that nanobionic approaches helped in crop improvement and monitoring of environment by inserting nanoparticles into plant cell by improving imaging.

Fang et al. (2017) have reported that glutathione has nanoparticles (Au-NPs) for acetylcholinesterase (AChE) activity detection by means of fluorescence and toxic and heavy metal Cd^{2+} in water samples. Application of nanosensors in agriculture is a promising tool which provides the assurance of development by monitoring soil and crop health. However, the large number of records of research in this area, regarding the performance of reliable nanosensors, is surprisingly insufficient in field, opening a window for research in future.

5 Application of Nanosensors in Agriculture

Nanosensors have more advantages in comparison with conventional sensors because of higher sensitivity, quick response, reliable results, large surface-to-volume ratio and high stability. Detection range is small in gram/mole range or lower than that which is found in several matrixes and facilitates fast electron transfer kit. Nanosensor-based system on global positioning level has been used for monitoring of cultivated fields at real time level in the growing season. All these applications assure the monitoring of crop growth at real time level and high-quality data which could be effective and further provide chances for management practices and ignoring large dose of agricultural inputs.

There are different metal nanomaterials such as quantum dots (QD), carbon nanotubes (CNT), gold nanoparticles (AuNP) and nanocomposites with polymers used in construction of nanosensors for the detection purpose of pesticides, insecticides, acting as a disease detection tool, providing smart agricultural practices, etc. (Zheng et al. 2011; Cesarino et al. 2012; Liu et al. 2012).

A. Nanosensors Provide Smart Agricultural Practices

Society is more dependent on the conventional agriculture practices and is transforming itself into smart agriculture in which the main contribution came from nanosensors, and it facilitates in progress of crop growth, detects pest attack on crop in field condition, detects diseases in various crops and reduces environmental stress (Chen and Yada 2011). Real-time monitoring with nanosensors prevents the use of fertilizer and pesticides and reduces environmental contaminants as well as the cost of the product.

Some of the activities included in smart agriculture are as follows:

(a) Fertilizer or pesticide delivery system facilitated by nanoformulations increases the wettability and dispersion of nutrients.
(b) Fertilizer or pesticide residues are detected by nanosensors.
(c) Disease incidence and crop growth were monitored by remote sensor systems.

B. Nanosensors Detect the Soil Humidity

Ganeshkumar et al. (2016) showed that nanofibers in one dimension made up of potassium niobate ($KNbO_3$) are sensing the humidity because of their large surface-to-volume ratio. Humidity nanosensors produce a result in the form of log value in linear form dependant on conductance versus relative humidity at the interval of two seconds. Results showed an increase in conductance from 10 − 10℧ to 10 − 6℧ for relative humidity range from 15 to 95% at room temperature.

C. Nanosensors and Crop Improvement

Bionic plants are developed through concise farming with the insertion of nanoparticles into the plant and chloroplast cells for imaging the presence of different objects in environment. Self-powering of plants is enhanced by communication of infrared devices or light sources (Ghorbanpour and Fahimirad 2017; Kwak et al. 2017). In one study, Giraldo et al. (2014) and Wong et al. (2016) reported that in in vivo conditions, single-walled carbon nanotubes (SWNTs) when inserted in plant system increase the photoabsorption. SWNTs suppress the reactive oxygen species (ROS) generation in chloroplast, and near-infrared fluorescence light-harvesting capacity is increased which results in photosynthetic efficiency and yield of plants. Hence, nanobionic approaches help in crop improvement and monitoring of environment.

D. Nanosensors Used for Herbicide Detection

Nanosensors composed of TiO_2 nanotubes were used for atrazine detection in soil reported by Yu et al. (2010). Chitosan composites and carbon nanotubes which are multiwalled were used in detection of methyl parathion in acetylcholinesterase enzyme modified with glassed electrode made up of carbon and detected in small amount in water and soil (Dong et al. 2013). Inhibitory effect of acetylcholinesterase enzyme was used for detection of methyl parathion. An amino-containing phosphorus such as glufosinate and glyphosate herbicide in soil was detected by nanofilm-modified pencil graphite electrode in range of 0.19–0.35 mg mL^{-1}, respectively.

Herbicide chlortoluron was detected by enzymatic nano-biosensor (Haddaoui and Raouafi 2015). The application of herbicide in agriculture provides prevention of growth of weed in cereal fields. The nanostructured method utilizing ZnO nanoparticles and modified carbon electrodes having screen-printed (SPCEs) which allows the detection of an activity of enzyme inhibition in tyrosinase and herbicide level of chlortoluron in part per billion (ppb) is also detected. This nano-biosensor has a 0.47 nano-mole (nM) detection limit. Herbicide chlortoluron induces tyrosinase inhibition in range from 1 to 100 nM.

E. **Detection of Pesticide and Insecticide by Aptamer-Based Nanosensor**

Aptamers are made up of peptide molecules or single-stranded nucleic acid having size less than 25 kDa with natural or artificial origin, also known as antibodies which are used for recognition element of aptasensors. Aptasensors are further used in pesticide and insecticide detection. They are also used for further detection of heavy metals such as Hg^{2+}, As^{3+} and Cu^{2+}. Even antibiotic kanamycin, tetracycline, oxytetracycline and cocaine were also detected by aptasensors. Acetamiprid in soil ranging from 75 nM to 7.5 µM is detected by nano-biosensor made up of nanoparticles with gold aptamer containing acetamiprid-binder.

Some pesticides such as monocrotophos and organophosphate were detected by an electrochemical biosensor including injection having novel flow (Norouzi 2017). Integrated results of chitosan–gold nanoparticle film are produced with technique called as fast Fourier transform continuous cyclic voltammetry (FFTRCCV). The purpose is to use chitosan–gold nanoparticles for increasing the immobilization level, and the results are obtained in less than 70 s, and further increased sensitivity has 10 nm detection limit; structure obtained was more stable having more than 50-day storage stability.

F. **Nanosensors in Insecticide Detection**

Another biosensor called as amperometric immunosensor was used for carbofuran detection which is a broad-spectrum insecticide used in agriculture. Gold nanoparticles are immobilized with monoclonal antibody specific to carbofuran on the glutathione. Carbon nanotubes are having multiwalled and sheet of grapheme made up with polyethyleneimine polymer–gold nanocomposites via self-assembly and modified on to the surface of a glass carbon electrode. And further, this antibody conjugates with gold nanoparticles detected by the immunosensing method in detection limit 0.03 ng mL^{-1}. This simultaneous immunological and electrochemical strategy provides highly reproducible, high stability, more specific and good regeneration capability nanosensors (Zhu et al. 2013). Another study on quantum dots (QDs) was done for methyl parathion detection by chronoamperometric sensor in the presence of substrate called as ATCl before and after inhibition with different concentrations of methyl parathion producing results variation in oxidation current which gives the concentration of methyl parathion involved in a reaction. QDs are nanocrystals known for their fluorescence spectra, full wavelength absorbance, high photo-stability and fluorescence emission in controlled manner. QDs have all these properties which provide them a property for imaging and sensing purpose. Construct prepared in the study was made up of ZnSe quantum dots attached with graphene–chitosan nanocomposites electrostatically and casted on a glassy carbon electrode, and acetylcholinesterase with mercaptophenyl boronic acid-functionalized was quantitatively detecting methyl parathion in 0.2 nM in the form of electrochemical signals.

G. **Nanosensors As Disease Detection Tool**

a. Nano-biosensor made up of chitosan and ZnO nanoparticles nanocomposite with electrode made up of gold is developed to detect fungal pathogen *Trichoderma harzianum* (Siddiquee and Suryani 2014).
b. *Polymyxa betae*, causal agent of necrotic yellow vein virus, which is detected by quantum dots consisting of fluorescence resonance energy transfer (FRET) nano-biosensor is used for identification of disease in sugar beet named as *Rhizomania* (Safarpour et al. 2012).
c. Bakhori et al. (2013) have reported that FRET is used for identification of *Ganoderma boninense* with oligonucleotide in which sensor is made up of deoxyribonucleic acid (DNA) probes and quantum dots.
d. Gold nanoparticle tagged with horse radish peroxidase for bacterial detection such as *Pantoae stewartii* (Zhao et al. 2014a, b).
e. Label-free gold nanorods were used for Odontoglossum ringspot virus detection and Cymbidium mosaic virus detection in 42 and 48 pg mL^{-1}, respectively.

f. A bacterial plant pathogen *Xanthomonas axonopodis* pv. vesicatoria in solanaceous crops was detected with fluorescent silica nanoprobes tagged with secondary antibody of goat anti-rabbit Ig (Yao et al. 2009).
g. Karnal bunt disease in wheat was detected with immunosensor of nano-gold (Singh et al. 2010).

H. Nanosensors Detect Nutrient concentration

Soil suffers from a loss of nutrient concentration, and it is important to analyse soil requirement for conditioning and productivity increase, and components which are in excess suffer from leaching. Some of the nanosensors which are used in nutrient detection are as follows:

Graphene oxide-based nanosensors were useful in nitrate detection, and nanosensors such as nanofibres made up of graphite oxide sheet and compound poly (3, 4-ethylene dioxythiophene) were for detection of nitrate (Pan et al. 2016; Ali et al. 2017).

I. Nanosensors Detect Fertilizer Activity

In the current society, fertilizer estimation is increasing with the help of nanosensors, further helping in cost management of fertilizers for reducing the pressure for farmer and saving fertilizers which are unutilized. Some of the nanoparticle-based biosensors were used to determine the urea, urease inhibition and urease activity which are as follows:

Urea, urease inhibition and urease activity were recognized by nanosensor made up of gold nanoparticle-3, 3′, 5, 5′-tetramethylbenzidine-H–O (Deng et al. 2016). Gold nanoparticle acts as a detection tool and produces yellow colour and has detection limit for recording urease activity (1.8 unit per L) in soil.

J. Nanosensors As An Agent for Promotion of Sustainable Agriculture

Nanofertilizers deliver nutrients to crops in the form of a product encapsulated with nanoparticle. Advantage of using nanofertlizers is reducing nitrogen loss due to emissions and leaching (De Rosa et al. 2010)

There are three ways of encapsulation:

(a) Nanoporous materials or nanotubes can contain nutrients or coating with thin film made up of polymers and delivering an emulsions or nanoparticles.
(b) Carbon nanotubes have penetrated in tomato seeds (Khodakovskaya et al. 2009).
(c) Nanoparticles made up of ZnO enter the ryegrass root tissue (Lin and Xing 2008).

Studies suggested that delivery system of nutrients explores the porous domains in nanoscale range on plant surfaces which release nutrients and prevent their changing state into gaseous or chemical forms whose further absorption cannot occur by plants. In order to attain the absorption, biosensor is equipped with nanofertilizers and allows controlled delivery of nutrients. Soil nutrient and environmental conditions also improves the quality of soil by reducing toxic effects caused by fertilizers.

K. Nanosensors in Regulation of Plant hormones

McLamore et al. (2010) demonstrated the use of MWCNTs helped in the study of plant growth by hormone regulation especially auxin and helped to understand the mechanism of plant roots acclimatization in the environment in marginal soils.

6 Conclusion

The use of nano-biosensors in agriculture enabled for improvement in detection capacity of microorganisms contaminants which are toxic and detect pesticide and insecticide residues. The support of nanomaterials to biosensor technology provides a better device, which can be handled easily and more sensitive and helps in improvement of detection speed. In addition, it has capability for sensing single analyte which gives the information of toxic contaminants present in agriculture. Sensing system increases the selective or specific method of detection for pathogens during antigen–antibody interactions.

Nano-biosensors are still in its development stage in rural small-scale farms, but the support of different type of nanomaterials is effective for biosensors because the cost is less, highly sensitive, user-friendly, high specificity and no technician requirement. Therefore, this nano-biosensor technology will be effective in increasing the crop production for fulfilling the increasing demands for food and provide novel devices for farms in rural or remote areas in order to give benefits for early monitoring of crop.

In a nutshell, the use of bio-nanosensors provides smart agricultural practices and has large number of applications in agriculture such as a detection tool for diseases for quick identification of pathogens and therefore help in managing plant diseases. It also helped in hormones delivery such as auxin and gibberellin with the help of multiwalled carbon nanotubes and promoting plant growth and detects activity of fertilizer, nutrient concentration, insecticides and pesticide residues.

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