CHAPTER

7

A Green Nano-Synthesis to Explore the Plant Microbe Interactions

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OUTLINE

7.1 INTRODUCTION

Green nanotechnology will be the foundation paradigm for next green revolution of India. India, an agricultural-based developing country, plays major role in shaping sustainable development goals (SDGs). Among the 17 goals of SDGs, agriculture has been codified at Goal 2 as Zero Hunger with the aim "end hunger, achieve food security and improved nutrition and promote sustainable agriculture." Various efforts have been carried out to achieve aim of zero hunger in India. Nanotechnology is an emerging and powerful tool for mankind that can play a vital role in an array of allied sectors in their upcoming year to solve big problems with nanoparticles. Nanotechnology is a boon to mankind because it is a powerful weapon for human welfare (Larue et al., 2014). Globally nanoparticles are increasingly used in many sectors of the economic area, raising interest in their production for the biological synthesis and environmental

New and Future Developments in Microbial Biotechnology and Bioengineering
https://doi.org/10.1016/B978-0-444-64191-5.00007-9

safety (Kadziński et al., 2018). Nanoparticles commonly synthesized by chemical and physical methods are often costly and eventually harmful to the environment (Makarov et al., 2014a, b). Numerous natural chemical substances act as a potent reducing agent, which has been used for the synthesis of nanoparticles, including the phytochemicals present in the plant extracts (Virkutyte and Varma, 2011 and Iravani, 2011). Similarly, several microorganisms such as algae fungi, bacteria, etc. also have many biological entities to transform organic metallic ions into metal nanoparticles, which is a relatively new and biggest unexplored field of research (Baker et al., 2013). A number of investigations are urgently required to develop nanoparticles with specific properties which are effective, affordable, and environmentally safe (Kumari et al., 2017a, b). This chapter describes some of the aspects related to green nanomaterial synthesis and their relationship with plant-microbe interactions.

7.2 GREEN SYNTHESIS OF NANOPARTICLES

Currently the title of "nanoparticles" has received specific interest in a wide range of fields. The term "nano" taken from the Greek word "nanos" means dwarf and denotes a measurement of one billionth (10^{-9}) of a meter (Narayanan and Sakthivel, 2010; Thakkar et al., 2010). There are two types of approaches that can be used in the synthesis of nanomaterials: "Top-down and bottom-up approaches." They are obtaining nanoscale materials by the fabrication of nanostructures and nanomaterials (Biswas et al., 2012). In the top-down procedure, macro- or microparticles are transformed into the nanoscale materials through the mechanical and fabrication technique. In the bottom-up approach, building complex from atomic level to nanoscale level through self-assembly mechanisms leads to the formation of nanomaterials (Iqbal et al., 2012). Nanoparticles are characterized by the analysis of size, morphology, and surface charge, using different advanced and sophisticated instruments, for example, atomic force microscopy (AFM), scanning electron microscopy (SEM), transmission electron microscopy (TEM), particles size distribution and dispersion of the nanoparticles, X-ray diffraction (XRD), X-ray photoelectron spectroscopy (XPS), and Fourier transform infrared spectroscopy (FTIR), etc. (Shaikh and Kaur, 2018).

Nanotechnology is a boon to mankind which established itself as a powerful weapon for human welfare (Larue et al., 2014). Global demands of nanoparticles are increasingly used in many sectors of the economic area, raising interest in their production for the biological synthesis and environmental safety (Kadziński et al., 2018). Nanoparticles commonly synthesized by chemical and physical methods are often costly and eventually harmful to the environment (Makarov et al., 2014a, b). There is an urgent need for wide varieties of biological research as an alternative, effective, affordable, and environmentally safe method for the development of nanoparticles with specific properties. Numerous natural chemical substances that act as a potent reducing agent for the synthesis of nanoparticles, including the phytoconstituents (e.g., tannins, flavonoids, terpenoids, saponins, glycosides, vitamins, proteins, peptides, biopolymers, etc.) present in the plant extracts were used for the green synthesis of nanoparticles (Virkutyte and Varma, 2011; Iravani, 2011). Similarly, microorganisms (e.g., algae fungi, bacteria, etc.) also have many biological entities to transform organic metallic ions into metal nanoparticles, which is a relatively new and biggest unexplored field of research (Baker et al., 2013; Singh, 2015a, b, c, d, 2016, 2018; Singh et al., 2016). Several plants, fungus, bacteria, actinomycetes, and agro waste have the potential to biosynthesize nanoparticles (Table 1; Fig. 1).

Biogenic nanoparticles synthesized by greener means have vast range of application. Biological synthesis of silver nanoparticles that was developed from potent fungal biocontrol agent *Trichoderma viride*, for the enhancement of antimicrobial potency, is urgently needed to search for a satisfactory biocandidate (Fig. 2) (Kumari et al., 2017a, b). A biogenic silver nanoparticle synthesized by plant extracts has been greener approach, which an important novel biotechnology (Mashwani et al., 2015).

7.3 NANOEMULSION SYNTHESIS

Nanoemulsion is the new advanced physiological form of emulsion by varying the physicochemical properties of macroemulsion and closely related colloidal dispersions (Mason et al., 2006). An emulsion is colloidal dispersion mixture of oil and aqueous phase while nanoemulsions with a size in the range of 10–200 nm are thermodynamically unstable but kinetically stable (McClements, 2012a, b). Small in size makes it optically transparent or turbid in appearance. The nanoemulsion hydrophilic components typically cover hydrophobic components. Nanoemulsion is a three-component system including oil, surfactant, and aqueous phase. Types of nanoemulsions depending on the

TABLE 1 Plants, Fungus, Bacteria, Actinomycetes, and Agro Waste Have the Potential to Biosynthesize Nanoparticles

Source of nanoparticles	Name of nanoparticles	Property and application of nanoparticles	References
Plants			
Azadirachta indica	Ag, Au	Ag; antibacterial activities against both gram positive (*Staphylococcus aureus*) and gram negative (*Escherichia coli*). Au; catalysis and biological sensor	Ahmed et al. (2016) and Huang et al. (2015)
Hibiscus rosa sinensis	Ag, Au	Au nanoparticles are bound to amine groups and the Ag nanoparticles to carboxylate ion groups	Philip (2009)
Acalypha indica	Ag	Antimicrobial activity against water borne pathogens, viz., *Escherichia coli* and *Vibrio cholerae*	Krishnaraj et al. (2010)
Artemisia nilagirica	Ag	Antimicrobial activity against gram negative bacteria	Vijayakumar et al. (2013)
Solanum lycopersicum	CdS nano crystals	Applications in medicine, bioassays, computing and photovoltaic's	Al-Shalabi et al. (2014)
Hordeum vulgare and *Rumex acetosa*	Iron oxide	Plant containing such constituents may be more efficacious for the green synthesis of iron nanoparticles	Makarov et al. (2014a, b)
Fungi			
Trichoderma viride and *Hypocrea lixii*	Ag, Au	Ag; Antibacterial activity, medical application etc. Au; pathogen control abilities, efficient biocatalyst	Kumari et al. (2017a, b) and Mishra et al. (2014)
Fusarium oxysporum	Au, Ag	Ag; Able to reduce *Leishmania amazonensis* promastigote and amastigote forms. Au; GNPs had high tendency of conjugation with antibiotics	Fanti et al. (2018) and Pourali et al. (2018)
Phanerochaete chrysosporium	Quantum dots	Bio-imaging and bio-labeling	Chen et al. (2014)
Penicillium sp.	Au	Large scale production to serve in industrial and medical fields	Magdi and Bhushan (2015)
Botrytis cinerea	Au	Rapid production	Castro et al. (2014);
Aspergillus flavus	Ag	Application for nano-factories	Jain et al. (2011)
Colletotrichum sp.	Au	Therapeutic applications	Sun et al. (2018)
Volvariella volvacea	Ag, Au	Therapeutic applications	Philip (2009)
Bacteria			
Rhodopseudomonas capsulata	Au	Gold nanowires that could be tuned for microelectronics, optoelectronics, nanoscale electronic devices, and other fields	He et al. (2008)
Bacillus sp.	Ag	Potential therapeutic applications	Golinska et al. (2014)
Serratia sp.	Ag	Pathogen control abilities, efficient biocatalyst	Mishra et al. (2014)
Escherichia coli	CdTe quantum dots	Broad bio-imaging and bio-labeling applications	Bao et al. (2010)
Algae			
Chlorococcum humicola	Ag	Biological metal recovery, bioremediation, bioleaching and biomineralization	Jena et al. (2013)
Yeast			
Extremophilic yeast	Ag, Au	Control label structure of Ag and Au.	Mourato et al. (2011)
Saccharomyces cerevisiae	Ag, Au, Se	Therapeutic application	Pereira et al. (2018) and Gilbert et al. (2018)

composition of the component are as follows: oil-in-water—when oil is dispersed in aqueous phase. Water-in-oil water droplets are dispersed in continuous oil phase (Fig. 3). Some researchers also stated a third category as bi-continuous nanoemulsion—oil and water droplets are involved in another system (Thakur et al., 2013). Surfactant and cosurfactant are used as utmost components as they provide stability to nanoemulsions (Jaiswal et al., 2015).

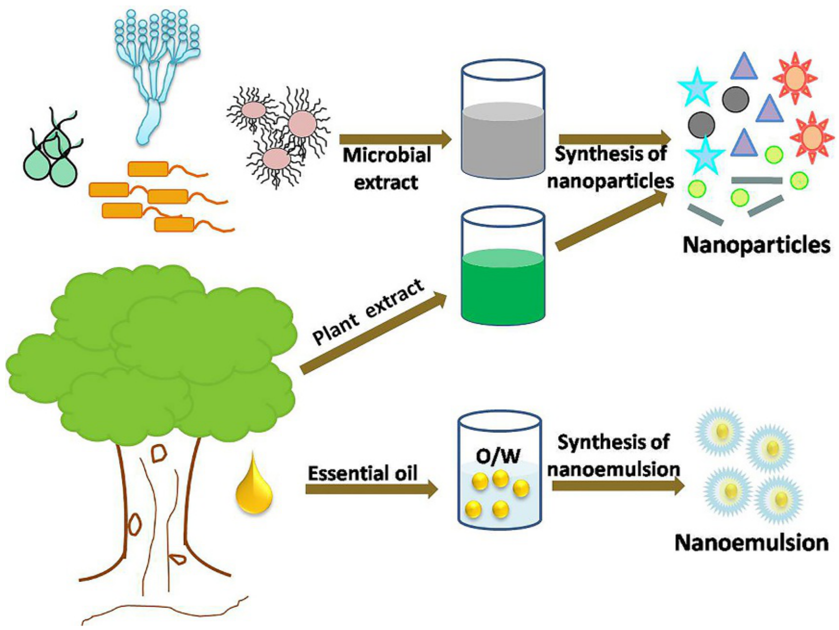

FIG. 1 Green synthesis of nanoparticles and nanoemulsion.

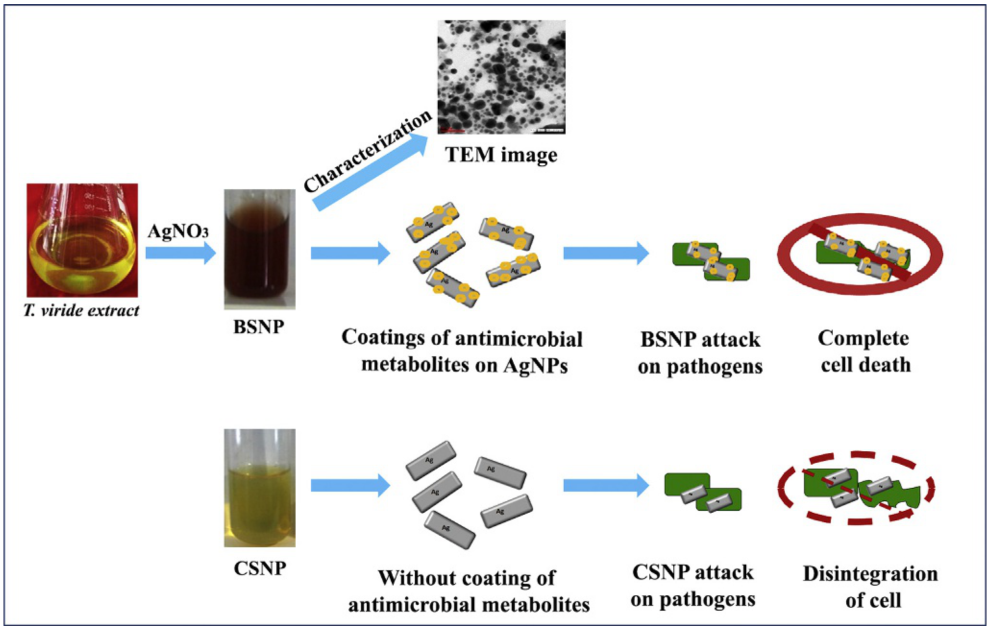

FIG. 2 Action mechanism of biosynthesized silver nanoparticles and citrate-stabilized silver nanoparticles against pathogens. *Adapted from Kumari, M., Shukla, S., Pandey, S., Giri, V.P., Bhatia, A., Tripathi, T., Kakkar, P., Nautiyal, C.S., Mishra, A., 2017a. Enhanced cellular internalization: a bactericidal mechanism more relative to biogenic nanoparticles than chemical counterparts. ACS Appl. Mate. Inter. 9, 4519–4533; Kumari, R., Singh, J.S., Singh, D.-P., 2017a. Biogenic synthesis and spatial distribution of silver nanoparticles in the legume mungbean plant* (Vigna radiata *L.). Plant Physiol. Biochem. 110, 158–166.*

Oil plays a pivotal role in nanoemulsion synthesis, which determines the functional property of nanoemulsion. As drug is soluble in oil phase, so it is a measurable step to select appropriate oil. For lipophilic drugs, O/W nanoemulsion is mostly preferred for loading whereas W/O is compatible for hydrophilic drugs (Azeem et al., 2009; Piazzini et al., 2017). Essential oils of herbal plants are well known for antimicrobial activity (Guerra-Rosas et al., 2017; Hammer et al., 1999; Gavini et al., 2005) and larvicidal activity can be used for nanoemulsion synthesis for enhancing activity due to nanotization of oil droplets. Apart from this, various oils have also been reported for skin nourishment, used in cosmetic coating on edible fruit and food, etc. (Table 2).

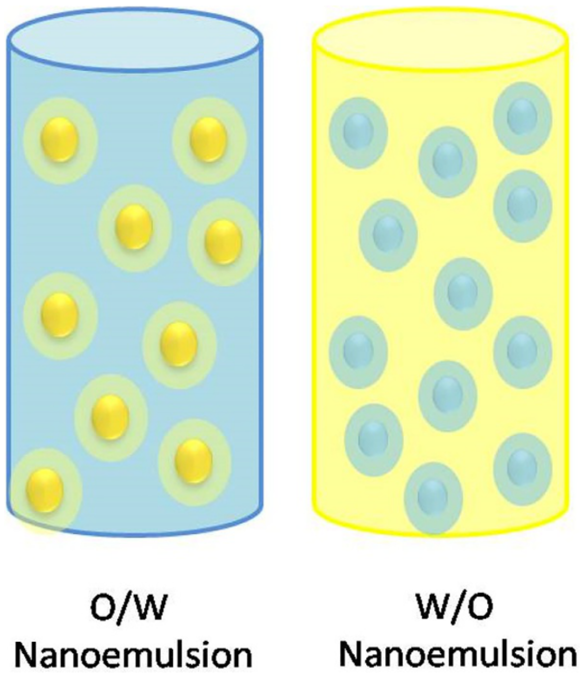

FIG. 3 Types of nanoemulsion: O/W nanoemulsion and W/O nanoemulsion.

TABLE 2 Synthesis of Nanoemulsion From Plant-Derived Oils, Method of Preparation and Their Application

Source of oil	Method of preparation	Properties/application	References
Phyllanthus emblica	Hot high-pressure homogenization	Drug delivery	Chaiittianan and Sripanidkulchai (2014)
Boswellia Serrata	High-speed homogenizer	Encapsulate drug for inflammatory disorder	Gohel et al. (2014)
Aniseed, peppermint and lemongrass	High-intensity ultrasonic	Pesticide	Pascual Villalobos et al. (2017)
Thymol	Sonication method	Antibacterial against pustules disease	Kumari et al. (2018)
Eucalyptus oil	Low-energy emulsification method	Antibacterial against *Listeria monocytogenes*	Sugumar et al. (2015)
Eucalyptus globulus	Ultrasonicator	Insecticidal activity of wheat weevil	Mossa et al. (2017)
Ocimum basilicum L	Sonicator	Larvicidal activity against *Aedes aegypti*	Ghosh et al. (2013)
Azadirachta indica	high-energy sonication	Larvicidal against *Culex quinquefasciatus*	Anjali et al. (2012)
Thymus daenensis	High-intensity ultrasound	*Escherichia coli*	Moghimi et al. (2016)
Jojoba oil	Sonication	Insecticidal Activity against *Sitophilus oryzae*	Sh et al. (2015)
Capsicum annuum L.	High-speed homogenizer	Encapsulation of drug	Pascual Pineda et al. (2015)
Vitex agnus-castus	Ultrasonication	Drug delivery	Piazzini et al. (2017)
Olive oil	Phase inversion temperature	Lipstick	Munawiroh et al. (2017)
Vegetable oil		Pesticidal activity against creeping foxglove, slender button weed (*D. ocimifolia*) and buffalo grass	Lim et al. (2013)

Continued

TABLE 2 Synthesis of Nanoemulsion From Plant-Derived Oils, Method of Preparation and Their Application—Cont'd

Source of oil	Method of preparation	Properties/application	References
Orange oil	Isothermal low-energy method	Delivery system in food industries	Chang and McClements (2014)
Aniseed	Homogenization	Antimicrobial activity against food-borne pathogens	Topuz et al. (2016)
Soybean oil	Boiling	Sporicidal activity against *Bacillus cereus* and *B. anthracis*	Hamouda et al. (1999)
Luteolin loaded emulsion	Phase inversion composition and sonication	Hair growth promotion activity	Shin et al. (2018)
Carapa guianensis (seeds); *Rosmarinus officinalis; Baccharis reticularia*	Low-energy method	Larvicidal activity against *Aedes aegypti*	Jesus et al. (2017), Duarte et al. (2015), and Botas et al. (2017)
Eucalyptus globulus; *Pterodon emarginatus*	Sonication; low-energy method	Larvicidal activity against *Culex quinquefasciatus*	Sugumar et al. (2014) and Oliveira et al. (2017)

They can be synthesized by various methods: ultrasonic emulsification, high-pressure homogenization, microfluidization, phase inversion temperature (PIT), spontaneous emulsification, and solvent displacement (Jaiswal et al., 2015). Recently a new method has been developed called bubble burst method (Gupta et al., 2016). The size of the droplet can affect the optical appearance, shelf life, tunable rheology, and stability (Becher, 2001). Broad range of oils are used in the synthesis of emulsion and the biophysical property of the emulsion make it more applicable in pharmaceutical, cosmetic, drug delivery, food industries, etc.

The surfactants are mainly classified into three categories: ionic, nonionic, and zwitter ionic (McClements, 2005):

1. *Ionic surfactant*—Negatively charged, needs positive agent, causes irritation (Sole et al., 2006).
2. *Nonionic surfactant*—Widely used, includes sugar ester, most acceptable, less toxicity, and causes irritation (McClements and Rao, 2011).
3. *Zwitter ionic surfactant*—Having two opposite charge on molecule, used with cosurfactant.

7.4 METHODS OF PREPARATION

Nanoemulsion synthesis is broadly classified into two categories: low energy and high energy (Tadros et al., 2004; Solans et al., 2005; Fryd and Mason, 2010).

7.4.1 High-Energy Methods

It requires high energy to convert the macroemulsions into nanoemulsion, this approach is regulated by an equilibrium between two opposite process droplet disruption and droplet coalescence (Jafari et al., 2008). For droplet disruption intense energies are required to convert it into nano droplets and this intense force should be much higher than restoring forces, which resist the droplet in spherical shape (Schubert et al., 2003; Schubert and Engel, 2004). High pressure homogenization, ultrasonication, microfluidization comes under these categories. In high-pressure homogenization process high-pressure pump is preferred over the macroemulsion system for conversion into tiny droplets by shear process (Floury et al., 2004). Sonication process creates turbulence by producing ultrasonic waves by sonicator probe (Jaiswal et al., 2015; Savardekar and Bajaj, 2016). Microfluidization method used by microfluidizer works on the principal the flowing of two streams through the separate channels, and then interacts in a separate interaction chamber. During the interaction, the drop experience high shear (Gothsch et al., 2011) and intense disruptive forces are generated which leads to smaller droplets (McClements and Rao, 2011; Jaiswal et al., 2015).

7.4.2 Low-Energy Approaches

In low-energy methods, system passes through a state of low interfacial tension (Gupta et al., 2016), requires less input energy. Phase inversion method begin with the synthesis of a W/O macroemulsion which is then converted to an O/W nanoemulsion by changing the factor which affects the HLB number (temperature/concentration of electrolyte) or changing the HLB number (by mixing of surfactant) at constant temperature (Mangale et al., 2015).

The PIT is a temperature-dependent process, initially all the three components are mixed in O/W macroemulsion at room temperature, when temperature increases gradually the surfactant becomes lipophilic, it gets solubilized in oil phase. O/W is converted into W/O emulsion when it cooled down (inversion of temperature) to room temperature. In phase inversion composition method a W/O emulsion is prepared at room temperature without the use of organic solvent and heat. In emulsion, water is continuously added, at inversion point the interfacial tension of the oil-water interface is very low, W/O emulsion is now converted into the O/W nanoemulsion with low-energy requirements (Gupta et al., 2016; Izquierdo et al., 2002).

There many other nanoemulsion preparation methods available, such as bubble bursting, evaporative ripening, and hydrogel method. In bubble bursting method, bubbles bursts at interface between air and emulsion (contains surfactant), which synthesize the nanotized oil droplets in water phase (Feng et al., 2014). The evaporating ripening method is mainly for the nanoemulsion synthesis of high viscous oils. Initially by high-pressure homogenizer volatile and non-volatile oils are mixed, and droplets are created. After heating the emulsion the volatile component in droplets evaporates and shrink, only high molecular part will be left (Fryd and Mason, 2010), this method causes particle aggregation which could be prevented by high shear forces (Patel and Joshi, 2012). The hydrogel technique is similar to the evaporating ripening method, solvent phase is miscible with the non-solvent part of the component.

7.5 PROPERTIES OF NANOEMULSION

Stability of nanoemulsion is controlled by several factors, viz., concentration of surfactant and cosurfactant, property of oil, method used for emulsification, and other additives involved in emulsion (Mason et al., 2006; Shafiq et al., 2007; Baboota et al., 2007). The basic property of nanoemulsion is its stability, after some time stability will be disturbed by some physicochemical mechanisms which include flocculation, Ostwald ripening, chemical degradation, and coalescence.

7.5.1 Transparency

Macroemulsions are opaque in appearance as the droplets have dimension similar to the wavelength of light ($r \approx \lambda$), they have tendency to scatter the light. Nanoemulsions contain mini droplets (10–100 nm), relatively smaller than the wavelength of visible light ($r << \lambda$), so nanoemulsions are transparent or slightly turbid in appearance (Mason et al., 2006; Tadros et al., 2004; McClements and Rao, 2011; Gupta et al., 2016).

7.5.2 Rheology

Rheological properties of nanoemulsions have changed than the macroemulsion due to small size droplets (McClements and Rao, 2011). Rheology can be controlled by the droplet size and dispersed volume. One can tune-up the behavior of nanoemulsion according to the application. Nanoemulsions have much stronger elasticity than macroemulsion. In cosmetic and food industries several gelling agents and depletion agents are used to change the viscosity of nanoemulsion according to the need of the product.

7.6 APPLICATION OF NANOTECHNOLOGY IN AGRICULTURE

Nanotechnology have been widely accepted by the several industries (biomedical, pharmaceutical, cosmetic, environmental pollution, and electronics) because of its biocompatibility, small droplets size, easily absorbing property, masking, and multi-targeted site (Fig. 4). In the last 10 years, green nanotechnology has achieved potential to be the reason for next “Green Revolution in India.”

Among the latest line of technological innovations, nanotechnology occupies a prominent position in transforming agriculture coining the term agri-nanotechnology. Nanotechnology has the potential to provide the innovations for a

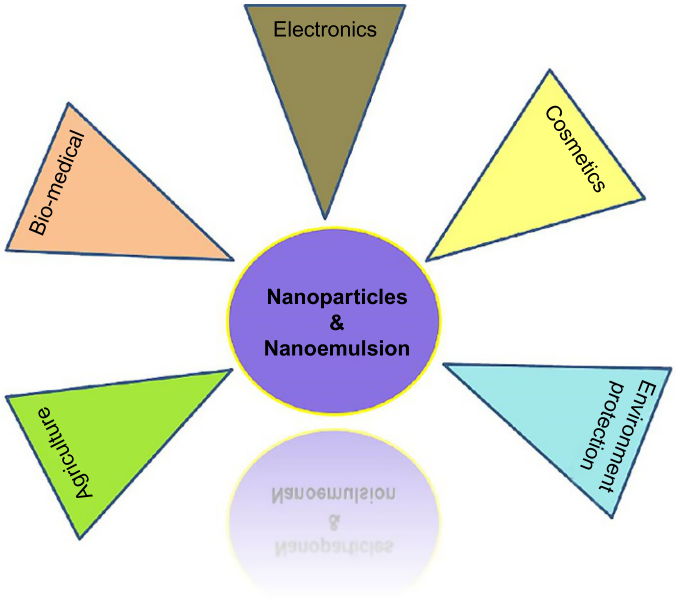

FIG. 4 Application of nanoparticles and nanoemulsion.

second green revolution in Indian agriculture (Sastry et al., 2007). Currently, such nanotechnologies have been reported to be used in the form of nanopesticides, nanosensors (Bouwmeester et al., 2009; Mukal et al., 2009), nanoscale adjuvants for pesticides (Bio-Based, 2010), smart delivery systems for nanoscale pesticides and fertilizers (Mukal et al., 2009; Ocsoy et al., 2013), and plant growth regulators (Khodakovskaya et al., 2013a, b; Sekhon, 2014).

7.6.1 Nanoparticles as Potential Plant Growth Promoters

Nanotechnology has recently entered into the field of plant growth promotion. Along with plant growth promoting bacteria (PGPB), with their multiple modes of action, it has emerged as new tool for promoting plant growth (Singh, 2013a, b, 2014). Water-soluble carbon nanotube showed enhancement of the growth rate of common gram (*Cicer arietinum*) plants by increasing the growth rate of root, shoot, and branching (Tripathi et al., 2011). It enhanced the water retention and absorption capacity in a nontoxic manner. Water-soluble nano onions obtained from wood wool also promoted the growth of gram by increasing the carbon, hydrogen, and nitrogen (CHN) contents of shoot and fruits (Sonkar et al., 2012). Metal nanoparticles including silver, zinc oxide, and titanium have also increased the plant growth rate in a concentration-dependent manner. Recently, nano-titanium (TNs) was shown to enhance the interaction between plants and colonization of growth-promoting rhizobacteria (PGPR) by forming a nanointerface (Timmusk et al., 2018). Phytomolecules coated with zinc oxide nanoparticles with supplementation of phosphate enhanced the plant growth in cotton (*Gossypium hirsutum* L.) by increasing the plant defense activities (Venkatachalam et al., 2017). Nanoparticles employ a multiple mode of actions including increased water absorption and retention capacity, minimizes the stress and increases the nutrient uptake efficiency. Iron oxide and sulfide nanoparticles have shown plant growth promotion even under low-nutrient uptake (Hu et al., 2017).

7.6.2 Nanoparticles as Potential Fertilizers

Fertilizers are essential components of modern agriculture because they provide essential plant nutrients. Nitrogen, phosphorous, calcium, and potassium are the major macroelements and iron and zinc are the major microelements required by the plant for its normal growth and development. A disturbing fact is that the fertilizer use efficiency is 20%–50% for nitrogen, 10%–25% for phosphorus and $<1\%$ for rock phosphate in alkaline calcareous soils. In such a critical situation, nanofertilizers can prove a very good answer to conventional fertilizers, binding to the nutrient in soil more thereby increasing the nutrient uptake efficiency, helping poor farmers to gain maximum yield applying minimum input (Table 3).

TABLE 3 Application of Nanofertilizers on Plants and Their Mode of Action

Type of nanofertilizer	Application on plants	Mode of action	References
MACRONUTRIENTS			
Carbon			
(CNT, graphene, fullereol, carbon nano-onions, carbon nanodots)	Tobacco, tomato, gram, barley, soyabean, corn; *Laphanussativus*	Upregulation of genes involved in cell division/cell wall formation and water transport and water channel proteins	Khodakovskaya et al. (2012), Khodakovskaya et al. (2013a, b), Lahiani et al. (2013), Park et al. (2014), and Tripathi et al. (2017)
Nitrogen			
Coating of urea with nano-ZnO (<100 nm) and nano-rock phosphate	–	Enhancing NUE efficiency	Kundu et al. (2018)
Chitosan NPK nanofertilizers	–	Nanoparticles were taken up and transported through phloem tissues	Abdel-Aziz et al. (2016)
Urea – HA nanohybrids	Wheat	Enhanced NUE efficiency	Chhowalla (2017)
Urea-hydroxyapatite Nanohybrids for slow release of Nitrogen	Rice	Slow release	Kottegoda et al. (2017)
Phosphate and calcium			
Calcium phosphate nanoparticles	Rice	Superadsorbent and water retention	Upadhyaya et al. (2017)
Slow release potassium silicate fertilizer	Rice	Enhanced availability	Wu and Liu (2007)
Apatite nanoparticles	Soyabean	Higher sustained P availability	Liu and Lal (2014)
Thermoplastic starch/urea (TPSUr) nanocomposites	–		Giroto et al. (2017)
Hydroxyapatite nanorod	Chickpea	Enhanced availability	Bala et al. (2014)
Hydroxyapatite and Goethite nanoparticles	–	Enhanced availability	Wang et al. (2015)
Potassium			
Potassium sulfate nanoparticles	Alfalfa (*Medicago sativa* L.)	Reducing electrolyte leakage, increasing catalase and proline content, and increasing antioxidant enzymes, activity	Patra et al. (2013)
Sulfur			
Sulfur nanoparticles	Mung (*Vignaradiata*)	Photosynthetic pigments (chlorophyll, carotene, and xanthophyll content) and mitochondrial stress indicator level (thiol)	Patra et al. (2013)
Sulfur nanoparticles	*Ocimumbasilicum*	Enhanced NUE	Alipour (2016)
Magnesium			
Mg nanoparticles	Wheat	Mobilization of native nutrient	Rathore and Tarafdar (2015)
Magnesium oxide nanoparticles	Maize	Enhanced chlorophyll content	Jayarambabu et al. (2016)
Magnesium oxide nanoparticles	Cluster bean	Enhanced chlorophyll content	Raliya et al. (2014)
MICRONUTRIENTS			
Manganese			
Manganese Nanoparticles	Mung bean	Potent Enhancer in Nitrogen Metabolism	Pradhan et al. (2014)

Continued

TABLE 3 Application of Nanofertilizers on Plants and Their Mode of Action—Cont'd

Type of nanofertilizer	Application on plants	Mode of action	References
Boron			
Boron coated on a metal nanoparticles	Potato	Availability of Boric acid	US patent
Boron nanofertilizer	Pomegranate	Enhanced availability	Davarpanah et al. (2016)
Iron			
Fe_3O_4 nanoparticles	Maize	Reducing the dose of Fe supplement for plants in the nano-form to increase the nutrient use efficiency	Elanchezhian et al. (2017)
Iron-sulfide nanoparticles	*Brassica juncea*	Improved iron content and redox status	Rawat et al. (2017)
Iron oxide nanoparticles	Peanut	Improved the availability of Fe to the plants	Rui et al. (2016)
Zero valent iron nanoparticles	Peanut	Uptake of NZVI by the plants	Li et al. (2015)
Silica			
Nanochisil	Maize	Increase in uptake of silica	Gumilar et al. (2017)
Nanosilica	Maize	Increase in uptake of silica	Karunakaran et al. (2013)
Nano-SiO_2	Tomato	Increased seed germination potential	Siddiqui and Al-Whaibi (2014)
Copper			
Copper nanoparticles	Piegon pea	Increased physiological responses	Shende et al. (2017)
Zinc			
Zinc nanoparticles	Pomegranate	Enhanced availability	Faizan et al. (2018)
Zinc oxide nanoparticles	Tomato	Increased photosynthetic efficiency	Mahajan et al. (2011)
Zinc oxide nanoparticles	Mung bean	Increase in shoot height	Raliya et al. (2015)
Titania			
TiO_2 nanoparticles	Mung Bean	Increased chlorophyll content and total protein	Rafique et al. (2015)
Titania nanoparticles	Wheat	Increased physiological parameters	Rafique et al. (2015)
TiO_2 nanoparticles	Broad beans	Saline Stress tolerance	Abdel Latef et al. (2018)
Cerium			
Cerium oxide nanoparticles	*Brassica napus*	Saline Stress tolerance	Rossi et al. (2016)
Cerium oxide nanoparticles	*Arabidopsis thaliana*	Enhanced physiological and molecular response	Ma et al. (2013)

Compared with amounts of nitrogen applied to soil, the nitrogen use efficiency (NUE) by crops is very low. Between 50% and 70% of the nitrogen applied using conventional fertilizers is lost owing to leaching in the form of water-soluble nitrates, emission of gaseous ammonia and nitrogen oxides. Nanoparticles have the potential to control the release of fertilizer based upon time and environmental conditions. One milligram of nanoparticles owing to their high surface to volume ratio can be as effective as 1 kg of bulk applied.

The greatest limiting factor for increasing world food production next to nitrogen is phosphorus. Efforts have been done to improve P uptake efficiency by plants through nanotizing the particular element. Similarly, to enhance the uptake of micronutrient such as zinc (Zn) and iron (Fe) nanotechnology can prove a great help. Other nanoparticles such as carbon nanotubes, titania nanoparticles have also shown a very good capability of plant growth promotion gaining the status of super fertilizers (Table 3). A wide range of slow and controlled release fertilizers of sulfur, urea, phosphate, and nitrogen are serving the farmers for efficient plant growth with the help of nanotechnology.

Dr. Norman Borlaug has also appreciated the role of nanotechnology in agriculture saying "Work should begin now on the next generation of fertilizer products using advanced techniques such as nanotechnology and molecular biology."

7.6.3 Nanoparticles as Potential Nanosensors

Sensors are devices which can detect the chemical, physical, or biological changes much rapidly than a human does. Nanosensors can also be defined as "A chemical, biological or physical sensor constructed using nanoscale components, usually microscopic or submicroscopic in size." They allow the detection of water and nutrient level, pest and insect attack, and plant stress due to biotic and abiotic factors (Subramanian et al., 2007). Using these nanosensors, farmers would be able to maximize their productivity by minimizing their inputs by indicating the nutrient or water status of crop plants over fine spatial and temporal scales, allowing the farmer to apply nutrients, water, or crop protection (insecticide, fungicide, or herbicide) only where necessary.

One example of such approach is to incorporate biosensor in a very thin polymer film (<100 nm). It would interact with the desired root signal and will change the permeability of the polymer making urea available to plant only in need (Monreal, 2012). Kaushal and Wani (2017) have reviewed the role of nanosensors in enhancing the crop production by protecting the plants from the plant pathogens. Earlier, Ansari et al. (2010) had constructed nanostructured zinc oxide platform for mycotoxin detection. Low-cost detection techniques including quantum dots, nanostructured platforms, nano-imaging, and nano-pore DNA sequencing tools have the potential to enhance sensitivity, specificity, and speed of the pathogen detection thus facilitating high-throughput sensing.

7.6.4 Nanoparticles as Potential Inhibitors of Plant Pathogens

Nanoparticles and nanoemulsions have attracted significant scientific interest as a new generation of antimicrobial agents because of increasing resistance of microbes toward traditional antibiotics and fungicides. The capability of nanoparticles to produce cellular damage by various means make it very difficult for microbes to acquire resistance against them (Cui et al., 2012).

In agriculture, every year pests destroy a hefty amount of crop. As per the data collected by Dhaliwal et al. (2010), pests cause 25% loss in rice, 5%–10% in wheat, 30% in pulses, 35% in oilseeds, 20% in sugar cane, and 50% in cotton in India. In an agriculture-based country like India, there is an urgent need to develop such technologies which can combat these effects without hampering the budget of poor farmers. Though traditional strategies like integrated pest management (IPM) and chemical pesticide such as DDT have been used for a long time in agricultural practices to control the pests, IPM being not very efficient and DDT being toxic to human and animals have not achieved the desired outcome. Therefore, nanotechnology is the way which can provide green and efficient alternatives for the management of insect pests in agriculture without harming the nature.

Nanopesticides are the metal nanoparticles itself or formulations with nanoparticles which are very toxic to bacteria and fungi at very much lower concentration than traditional chemical pesticides or water- or oil-based nanoemulsions having suspension of pesticides. These nanoparticles suspensions ensure the enhanced solubility of pesticide due to increased surface contact area and long slow sustained release thereby reducing the wastage of pesticides. This technology certainly provides an edge over others to lower down the cost spent on pesticides and also helps to keep the environment clean (Rai and Ingle, 2012; Guerra-Rosas et al., 2017).

Companies are using water- or oil-based nanoemulsions of nanoparticles having uniform suspension of pesticide or herbicide nanoparticles of 200–400 nm (Rickman et al., 2003). One nano-surfactant based on soybean micelles claims to make glyphosate-resistant crops susceptible to glyphosate when it is applied with the nanotechnology-derived surfactant (Bio-Based, 2010). Surface charged silver nanoparticles (SNP), modified hydrophobic nano-silica (3–5 nm), (Ulrichs et al., 2005), aluminum oxide (ANP), zinc oxide, and titanium dioxide in the control of rice weevil and grasserie disease in silkworm (*Bombyx mori*) caused by *Sitophilus oryzae* and baculo virus BmNPV (*B. mori* nuclear polyhedrosis virus), (Goswami et al., 2010), antifungal activity of SNP against the fungus *Raffaelea* sp., which was responsible for the mortality of a large number of oak trees in Korea are some examples of using metal nanoparticles as pesticides. Gajbhiye et al. (2009) have also reported the antifungal activity of silver nanoparticles against most important plant pathogenic fungi like *Fusarium* and *Phoma*. Biosynthesized silver nanoparticles have shown their enhanced

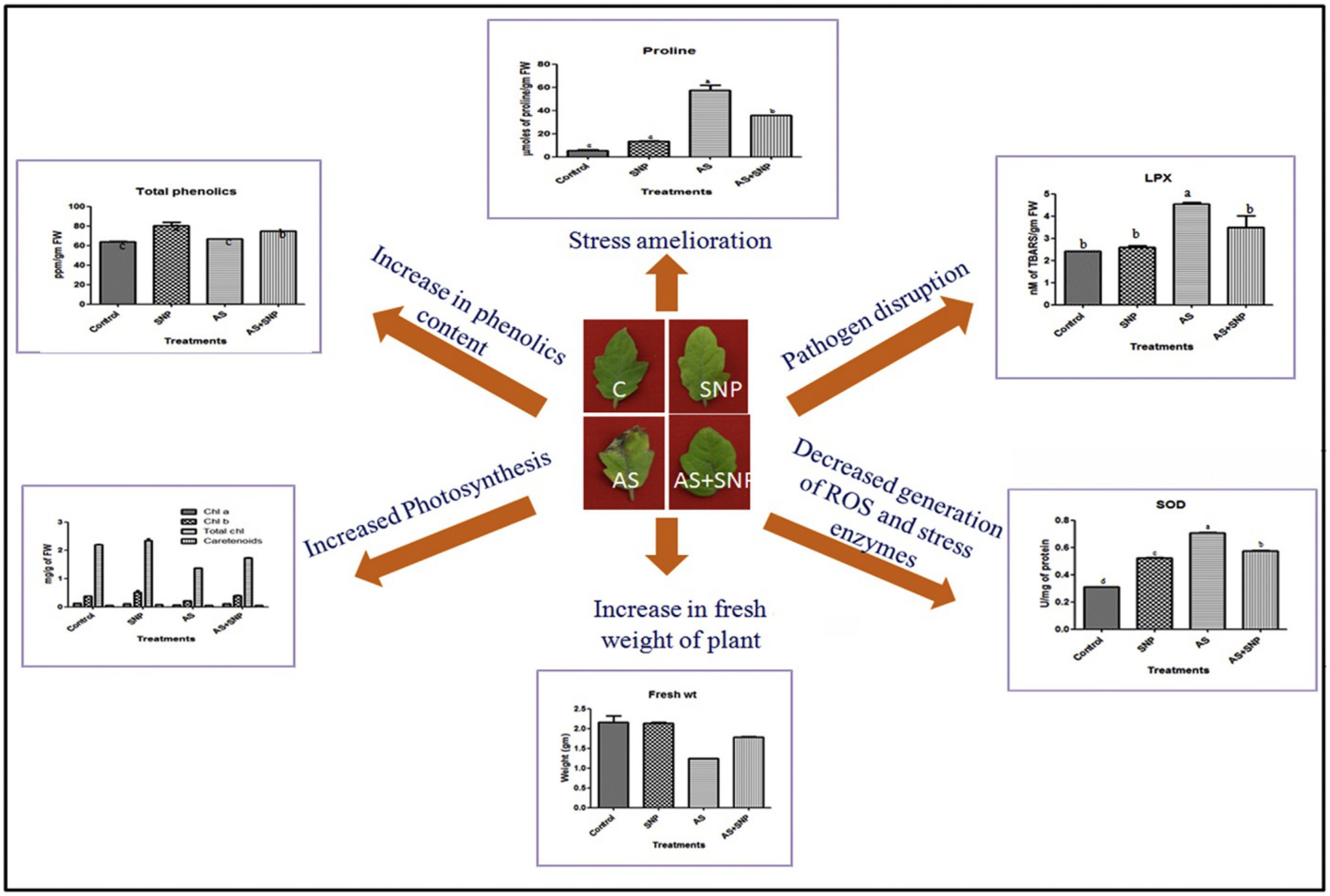

FIG. 5 Mechanisms employed by biosynthesized silver nanoparticles against *Alternaria brassicicola* in host plant tomato. *Adapted from Kumari, M., Shukla, S., Pandey, S., Giri, V.P., Bhatia, A., Tripathi, T., Kakkar, P., Nautiyal, C.S., Mishra, A., 2017a. Enhanced cellular internalization: a bactericidal mechanism more relative to biogenic nanoparticles than chemical counterparts. ACS Appl. Mate. Inter. 9, 4519–4533; Kumari, R., Singh, J.S., Singh, D.P., 2017a. Biogenic synthesis and spatial distribution of silver nanoparticles in the legume mungbean plant* (Vigna radiata *L.). Plant Physiol. Biochem. 110, 158–166.*

antimicrobial properties against phytopathogens *Pseudomonas syringae, Xanthomonas oryzae, Bacillus thuringiensis. Rhizoctonia bataticola, Aspergillus niger, Colletotrichum coccodes, Monilinia* sp., and *Pyricularia* sp. (Velmurugan et al., 2013; Papaiah et al., 2014; Siddiqui et al., 2018). Kumari et al. (2017a, b) have also reported the antifungal activity of silver nanoparticles against most important plant pathogenic fungi like *Fusarium* and *Alternaria* by increasing plant primary defense and directly killing the pathogens (Fig. 5). However, the literature available on this topic brings to a close conclusion that only a few researchers all over the world are working in this area, and hence, there is a pressing need to apply nanotechnology and this warrants detailed study in this field.

7.6.5 Plant-Pathogen Nanoparticle Interactions

Understanding the interactions between engineered nanomaterials and plants is crucial in comprehending the impact of nanotechnology on the plant disease treatment (Khodakovskaya et al., 2012). However there is dearth of knowledge about the plant's response toward nanoparticles acting as pathogen control in plant. A thorough understanding of the effects at physiological, morphological and molecular level of plants induced by the nano-sized engineered materials to prevent them from disease is a crucial part of research which has remained untouched yet. Jo et al. (2009), in their studies observed that silver nanoparticles demonstrated great potential in controlling spore-producing fungal plant pathogens. Growth chamber inoculation assays resulted in significant reduction of spore forming *Bipolaris sorokiniana* and *Magnaporthe grisea* on perennial ryegrass (*Lolium perenne*) with an application at 3h before spore inoculation. Similarly, in field trials with pepper, the inhibition of pepper anthracnose pathogen *Colletotrichum* sp. was achieved by application of silver nanoparticles before disease outbreak on the plants (Lamsal et al., 2011). Earlier, the same group of Lamsal et al. (2010) demonstrated the inhibitory effects of silver nanoparticles against powdery mildews on cucumber and pumpkin when applied at a concentration of 100 ppm. Chu et al. (2012) observed that pre-treatment of *Arabidopsis thaliana* with silver nanoparticles induced more pathogen resistance to the virulent pathogen *P. syringae*pv. Tomato DC3000 (Pst) and enhanced defense responses. Ocsoy et al. (2013) demonstrated application of silver and grapheme oxide composite on tomato transplants in reduction of the severity of bacterial spot disease with no phytotoxicity. Similarly, Mishra et al. (2014) found application of bio-fabricated silver nanoparticles as strong fungicide against *B. sorokiniana* causing spot blotch disease in wheat.

7.6.6 Cellular Uptake of Nanoparticles in Plants and Their Potential Effects

The fate of nanoparticles inside plant cell and their transport is also one of the subjects of curiosity which needs to be explored further. Interactions between plants and nanoparticles deserve more in depth investigation on many fronts, such as uptake potential of different plant species, mechanisms of uptake and translocation and the interactions between the particles with plant tissue at the cellular level (Zhu et al., 2008). Zhang et al. (2011) claimed their study to be the first, reporting uptake and distribution of metal oxide nanoparticles in plants where they observed uptake and distribution characteristics of two types of ceria nanoparticles with sizes of 7 and 25 nm in cucumber roots in dose-dependent manner. Vacuole, cell wall and rough endoplasmic reticulum were found to be the potential organelles for deposition of silver nanoparticles in *Erucasativa* (Vannini et al., 2013). Krishnaraj et al. (2012) demonstrated in their study that, biologically synthesized AgNPs interaction on the growth and metabolism of *Bacopa monnieri* could enhance the plant immune response which can further be applied to control several plant diseases and pathogen attacks.

7.6.7 Omics Approach in Studying Plant-Pathogen Nanoparticle Interactions

Omics approach refers to collective characterization and quantification of pools of biological molecules that translate into the structure, function, and dynamics of an organism(s). It includes field of study in Biology which ends with omics, viz., transcriptomics, proteomics and metabolomics. Omics approach has widely been used to study system biology in response to various biotic and abiotic factors. It has shown its remarked application in study of plant pathosystem (Pochon et al., 2012; Peyraud et al., 2018); stress responses of plant and bacteria (Ghosh and Xu, 2014) and response of plants to metal stress (Ruotolo et al., 2018). Despite the tremendous use of nanoparticles as new generation of antimicrobial agents, their mechanism to do so and their role in tripartite interaction of plant, pathogen and nanoparticles largely remains unknown yet. To get an insight into the effect of nanoparticles on plant machinery when they are applied as bio-control, it is necessary to get acquainted with their changes associated at gene, protein and metabolic levels by using omics approach.

7.6.8 Transcriptomic Profiling

The transcriptome is the set of all RNA molecules, including mRNA, rRNA, tRNA, and other non-coding RNA produced in one or a population of cells. In plants, nearly every cell contains the same genome and thus the same genes. However, pattern of gene expression is different in different cells because of physical, biochemical, and developmental variations. Thus, by collecting and comparing transcriptomes of different types of cells or tissues, under different conditions and treatments, a deeper understanding can be gained of what constitutes a specific cell type and how changes in transcriptional activity may reflect or contribute to disease.

Though, reports on transcriptomic response of plant toward plant-pathogen—nanoparticle interactions remains unexplored yet, some reports are available on trancriptomic changes of plants in pathogen attack (Ruotolo et al., 2018). Mukherjee et al. (2009) deciphered a compatible interaction of *Alternaria brassicicola* with *A. thaliana* ecotype DiG. They found that several known pathogenesis-related (PR) genes were upregulated in both compatible and incompatible interactions. PR1 and a monooxygenase gene MO1 were preferentially upregulated in the compatible interaction while GLIP1, which encodes a secreted lipase, and DIOX1, a pathogen response-related dioxygenase, were preferentially upregulated in the incompatible interaction. Plant-silver nanoparticle interactions have been studied by Pillai et al. (2014); they found that the cells mount a defense response to combat oxidative stress and to eliminate silver via efflux transporters at concentrations 10, 100, 200, and 500 nM. Transcriptome analysis in *A. thaliana* after exposure to AgNP, TiO_2 nanoparticles, and carbon nanotubes showed that exposure to NP triggers plant defense responses and activates mechanisms involved in both abiotic and biotic stress responses (Garcia-sanchez and Cristobal, 2013). Syu et al. (2014) studied the impacts of size and shape of silver nanoparticles on *Arabidopsis* plant growth and gene expression. They observed that AgNPs induced bifacial effects on plant growth, significantly inhibiting ethylene perception and increase root growth. They also observed that the phenotypic and genetic changes in *Arabidopsis* were highly correlated with nanoparticle morphology and size.

7.6.9 Proteomic Profiling

The proteome is defined as the complete protein complement of a genome. Similar to other omics approaches, proteomics has still not been used to study plant-pathogen nanoparticle interactions. Mukherjee et al. (2010) have observed the proteomic responses of *A. thaliana* to infection with *A. brassicicola*. They found upregulation of pathogen-related protein PR4, a glycosyl hydrolase, and the antifungal protein osmotin. Two members of the Arabidopsis glutathione S-transferase (GST) family increased in abundance in infected leaves. Vannini et al. (2013) used proteomic approach to study molecular responses of *E. sativa* exposed to silver nanoparticles or silver nitrate for the very first time. They observed that silver nanoparticles and $AgNO_3$ showed low level of overlap of differentially expressed proteins. Only the silver nanoparticles exposure caused the alteration of some proteins related to the endoplasmic reticulum and vacuole indicating these two organelles as targets of the AgNPs action. Yasmeen et al. (2018) studied proteomic analysis of shoot in stress-tolerant wheat varieties on copper nanoparticle exposure and found that protein abundance related to glycolysis and tricarboxylic acid cycle in wheat varieties was affected after exposure to nanoparticle.

7.6.10 Metabolomic Profiling

Metabolomics is a discipline of biochemistry concerning the scientific study of unique chemical fingerprints that specific cellular processes leave behind, the study of their small-molecule metabolite profiles. For an integrative approach toward systems biology it is essential to include metabolic data because changes in metabolites are most closely connected to the observed phenotype of an organism. Recently, a lot has been studied regarding plant-pathogen interactions using advanced technologies, such as gas chromatography mass spectrometry (GC–MS), liquid chromatography mass spectrometry (LC–MS), FTIR, and nuclear magnetic resonance (NMR) spectroscopy (Cleary et al., 2014). Metabolic profiling of plant-nanoparticle interaction is in nascent stage till now. After the treatment of bitter melon with carbon-based nanoparticles, the phytomedicine content of cucurbitacin-B, lycopene, charantin, and insulin content increased significantly (Kole et al., 2013). The metabolomic responses of plant after multiple modes of treatment with ceria oxide nanoparticles were also studied by Salehi et al. (2018). In their study, they found that metabolomics and proteomic changes are highly dependent on the mode of exposure and dosage of nanoparticles. To get an insight into the tripartite interaction of plant-pathogen nanoparticles, it is necessary to get acquainted with metabolic changes taking place during the above interactions.

7.7 NANOEMULSION IN AGRICULTURE

Nanoemulsion opened a vista by providing multidisciplinary action mechanism to agriculture. Although nanoemulsion technology still needs a death of knowledge in agriculture to overcome the lacuna of herbal-based solution that will apply in agricultural field without fear of bioaccumulation and biomagnifications. Herbal oil-based nanoemulsion is majorly used as pesticide, larvicide, and encapsulation of herbicide/pesticide/fungicide.

7.7.1 Antimicrobial Agents

Nanoemulsion of thymol shows plant growth promotion and antibacterial activity against bacterial pustule disease caused by *X. axonopodis* pv. *glycine* in soyabean (Kumari et al., 2018) with saponin, as surfactant of *Quillaja* tree. Thymol with saponin shows increase in activity due to nanotized droplet size and in combination with targets in multidirectional way, viz., easily penetrate through bacterial membrane, lipophilic moiety of saponin increase the cell lysis (Wojciechowski et al., 2014). Topuz et al. (2016) evaluated the antimicrobial activity of bulk and nanoemulsion of anise oil against *Listeria monocytogenes* and *Escherichia coli*. There might be possibility of interaction of microbial cell membrane with volatile molecules having more surface area, more interfacial area is present than bulk molecule (Salvia-Trujillo et al., 2014). Some reports also showed destabilization of volatile compounds at interfacial layer from surfactant molecules after which it get attached with the microbial cell. According to Kumari et al. (2018), thymol nanoemulsion induces plant growth by increasing plant height, root length, fresh weight, and number of nodules/pods. There is lacuna of research need to understand the role and mechanism of nanoemulsion in plant growth promotion.

7.7.2 Larvicidal, Pesticidal, and Herbicidal Activity

Oil-in-water nanoemulsion formulation is in practice as pesticide delivery. Oil-based nanoemulsions have potential itself or encapsulate the bioactive compound to control the larvae of arthropods (Anjali et al., 2012; Jesus et al., 2017; Duarte et al., 2015; Botas et al., 2017; Sugumar et al., 2014; Oliveira et al., 2017) most populated animal in world.

Nanoemulsions have been evolving as nanopesticides due to its bioactivity and nanotized property of oil phase. Various efforts have been carried out to control the pest in agricultural pest, a major factor for crop loss. Many lipophilic (glyphosate) nanoemulsions of fatty acid methyl esters in controlling pest (Jiang et al., 2012; Lim et al., 2013). Cotton bollworms, a devastating pest infest on cotton plants, nanoemulsion of eucalyptus oil, especially targets on the newly hatched larvae of *Pectinophora gossypiella* and *Earias insulana*. Eucalyptus oil also have broad range of insecticidal activity against *Tribolium castaneum*, *Callosobruchus maculatus*, *Rhyzopertha dominica*, *Oryzaephilus surinamensis*, *S. oryzae*, and *Sitophilus granarius* (Mossa et al., 2017). Sh et al. (2015) observed the insecticidal activity of jojoba seed oil nanoemulsion against wheat pest *S. oryzae*. Size of oil droplets have reduced as a result it can easily penetrate cuticle layer of insect and also the mortality of adult insect is directly proportional to oil concentration. After harvesting, insect pest also damages the crop in stored houses. Choupanian et al. (2017) synthesized nanoemulsion by using neem oil, effective against *S. oryzae* (L.) and *T. castaneum* (Herbst), which will be an herbal alternative to pesticide in ware houses. Organic pesticides for sustainable development in agriculture promote the use of organic pesticides by synthesizing nanoemulsion of *Manilkara subsericea* extract (Fernandes et al., 2014). Du et al. (2016) loaded the β-cypermethrin water-insoluble pesticide with oil-in-water emulsion and observed the activity of pesticide.

Nanoemulsion technology has been further applied for successful control of herbs and weeds. Jiang et al. (2012) observed water-soluble glyphosate isopropylamine (IPA) nanoemulsion formulations against *Eleusine indica.* Macro- and nanoemulsions of Thyme and Marjoram essential oils have also shown the herbicidal activity on seeds and seedling of *Convolvulus arvensis* and *Setaria viridis*. For organic agricultural system essential oil of *Satureja hortensis* has been used as herbicide by Hazrati et al. (2017) for controlling weeds.

7.8 CONCLUSIONS AND FUTURE PROSPECTS

India is an agriculture-based country which provides employment to over 60% of the population and contributes to about 18% of the total GDP in Indian economy (Singh, 2012). Nano-science is a wonderful mixture of science, engineering, and technology offering a myriad of applications in every aspect of human life including agriculture. Biosynthesis of nanoparticles and nanoemulsions by plant and microbes has gained a significant interest because of being economical and eco-friendly. Both nanoparticles and nanoemulsions bear a great potential to act as potent antimicrobial agents against a range of plant pathogens. Further, nanoparticles have shown their application in plant growth promotion, as nanofertilizers and nanosensors. Despite multifarious uses of nanoparticles and nanoemulsions in agriculture, knowledge about regulation of transcriptome, proteome, and metabolome of plant during tripartite interactions of plant pathogen and nanoparticles is still in nascent stage which needs to be deciphered. Although nanotechnology has given birth to a great enthusiasm for developing countries regarding their application in agriculture, some bottlenecks are still to overcome. Risks associated with the use of particles, intellectual property rights are some constraints which needs to be solved as soon as possible to let the nanotechnology ignite a new flame under which smiling faces of poor can be seen.

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