CHAPTER

25 Microbially synthesized nanoparticles: A promising future for insecticidal efficacy studies

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1 Introduction

After the serendipitous discovery of penicillin antibiotics in 1929, the emergence of microbes and its products came into the limelight for the welfare of human beings and the environment (Gaynes, 2017). The role of microbes in this globe is inevitable, which plays an important role in maintaining the ecosystem, healthcare, livestock management, food products, feeder, cosmetics, industrial enzymes, amino acids, vitamins, antibiotics, alcohol, beverages, pesticides, herbicides, insecticides, and sensors (Mahmood and Mahmood, 2015). It is uncertain for humans to live without microbes; because they are the only source for humans for maintaining the existence of life.

Microbes *Corynebacterium, Brevibacterium, Escherichia coli, Propionibacterium, Pseudomonas, Aspergillus, Lactobacillus, Rhizopus, Aspergillus, Bacillus, Corynebacterium glutamicum, Brevibacterium, Corynebacterium, Micrococcus, Microbacterium, Eremothecium ashbyii, Ashbya gossypi, Corynebacterium ammoniagenes, Propionibacterium shermanii, Pseudomonas denitrificans, Blakeslea trispora, Phycomyces blakesleeanus, Mucor circinelloides, Rhodotorula* spp., *Choanephora cucurbitarum yeasts Candida catenula, C. guilliermondii, C. tropicalis, Yarrowia lipolytica, Aspergillus, A. niger, A. wentii, Acctinobacillus succinogenes, Torulopsis glabrata,* and *Candida glycerinogenes*. The above-mentioned microbes have been intensively utilized in various therapeutic, industrial applications and other processes for well-being of humans and the environment (Singh et al., 2017).

Microbial metabolites are classified as primary and secondary metabolites; primary metabolites (amino acids, nucleotides, proteins, enzymes) involved in the growth, survival, reproduction of microorganisms. While secondary metabolites (organic compounds), were produced at stationary phases of growth that are not involved in the normal function of microorganisms. These secondary metabolites are largely utilized by research fraternities for value-added products such as antibiotics,

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antiparasitic agents, antinematicidal, anticancer, immunosuppressive, antiinflammatory, food preservatives, chemical catalysts, antimosquitocidal, and others (Demain, 1999). But the production, optimization of various factors for increased production and downstream process for recovery of secondary metabolites is a bottleneck factor. Through genetic engineering, the approach will solve the increased production of metabolites but that is also remains challenged in certain species.

An amalgamation of these secondary metabolites with new existing technology like nanotechnology is needed for scientific researchers to fabricate innovative drugs for emerging infectious and noninfectious diseases. It is well established that plants mediate the synthesis of metallic/metal oxide nanoparticles using a one-pot method and its application in a broad perspective in biological sciences (Khandel et al., 2018). But in microbial mediated synthesis burgeoning research is perceived to manipulate the metal nanoparticles production through intracellular/extracellular enzymes; microbes inherently possess the characteristics of accumulation metals into metal ions through enzymes (Banerjee et al., 2018). This phenomenon process is utilized by researchers to fabricate desired metal/metal oxide nanoparticles by microbes. The synthesized nanoparticles have great potential in biomedical applications. Henceforth, this chapter exclusively focuses on the microbial mediated synthesis of nanoparticles and its insecticidal efficacy.

2 Types of nanoparticles

Nanoparticles were generically classified based on the size, shape, and chemical properties (Fig. 1).

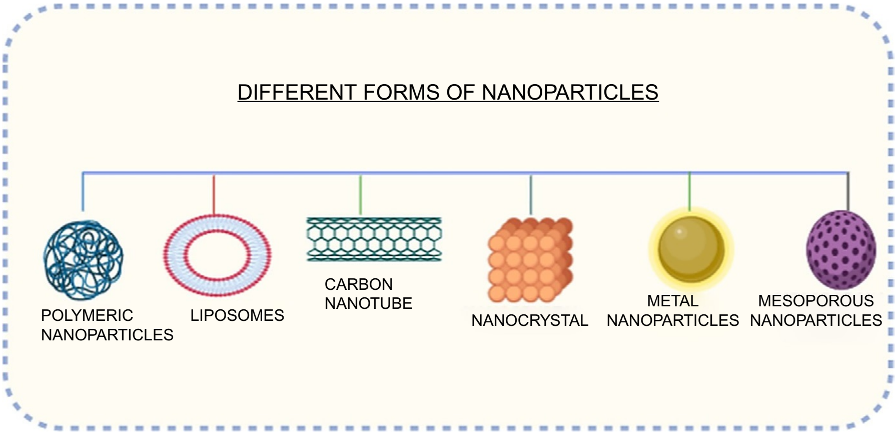

FIG. 1

Different forms of nanoparticles.

- Carbon NPs
- Polymer NPs
- Liposomes
- Semiconductor NPs
- Ceramics NPs
- Metal NPs

Each nanomaterial is multifaceted one; possessing physicochemical properties with broad-spectrum applications.

3 Synthesis of nanoparticles

The synthesis of nanoparticles is achieved by two different approaches such as the "top-up" and "bottom-down" approach (Fig. 2).

The top-down approach is a systemic method used to leach out the bulk solid materials bit by bit into fine generated powdered form. The production of nanoparticles is highly efficient producing monodispersed nanoparticles with discrete size and shape. But the physical method requires high energy, mechanical pressure, thermal energy, electrical energy to cause solid material to abrasion leading to a fine generation of nanoparticles (Iravani et al., 2014). The above method produces nanoparticles with imperfection in size and shape, such nanomaterials would have a significant impact on the applications.

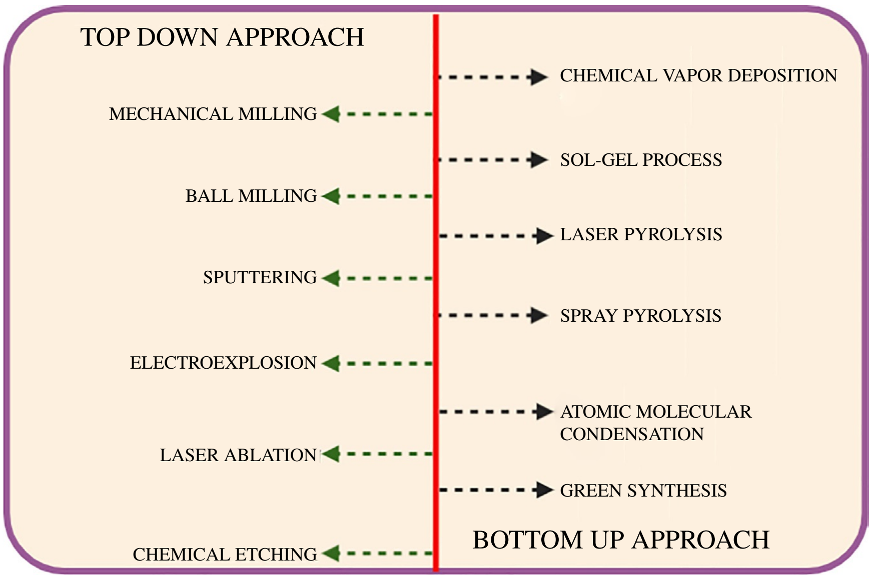

FIG. 2

Different approaches for synthesis of nanoparticles.

The bottom-up approach is one of the methods for nanoparticle synthesis. In this approach, nanomaterials were synthesized from the bottom; atom-by-atom, molecule by molecule; the atoms or molecule self-assemble into a concrete structure with high stability. The bottom-up approach is a commonly used method for nanoparticle synthesis; it can produce monodispersed nanoparticles with uniform size and shape (Wang and Xia, 2004). The synthesis method involves hazardous chemicals, reagents, solvents; energy and other factors that hamper the nanomaterials for biological applications and in the environment also. In Fig. 1, the bottom-up approach is listed; in this approach, a new form of synthesis referred to as green synthesis is gaining noble popularity among the research fraternity due to the ease process and having wide applications in health care and environment management.

3.1 Green synthesis of nanoparticles

Green synthesis of nanoparticles is a new perspective in the synthesis of nanoparticles employing the bioresources plants, bacterium, yeast, actinomycetes, fungi, and algae (Fig. 3).

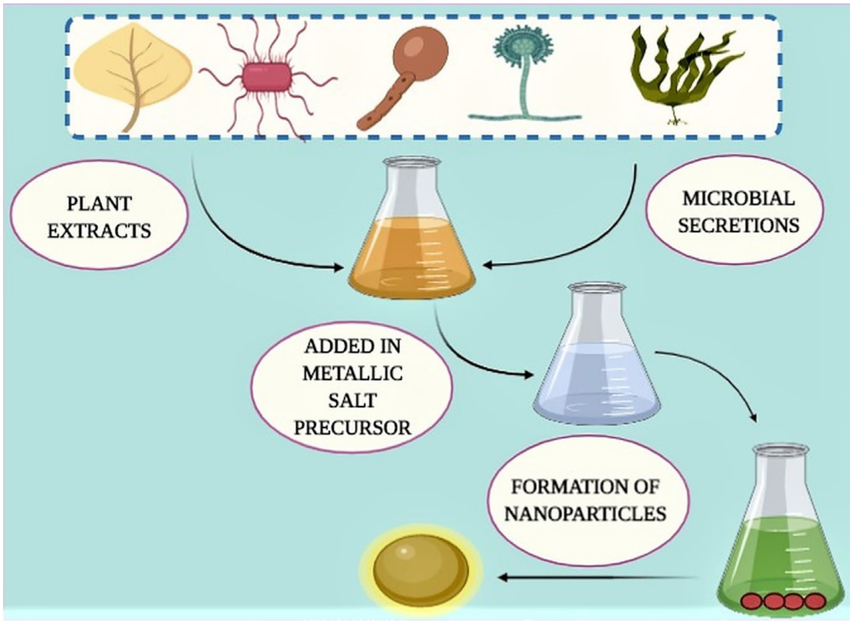

FIG. 3

Biological synthesis of nanoparticles.

3.2 Plant-mediated nanoparticles

Nature contains a plethora of plants having myriad therapeutic activities. Since from ancient medicine practices, the utilization of plants and its decoction is intensively practiced for ailments (Alamgir, 2017). Later, plants/decoction combined with metals known as Bhasma was practiced in Indian, Chinese, and Egyptian ayurvedic medicine. These formulations cure any kind of infectious and noninfectious diseases (Pal et al., 2014). But howsoever, without any holistic knowledge, the peoples started this treatment universally. Later, with the advance of science and technology, this Bhasma is now redefined with a new dimension as metallic nanoparticles synthesized from plant extracts.

Plant-mediated nanoparticles is a rapid procedure where a metal salt is synthesized with plant extract within a stipulated time frame. Plant metal nanoparticles are an eco-friendly approach of nanoparticles where the plant extracts utilized as a reducing and stabilizing agent in the synthesis process (Singh et al., 2018). The synthesis method is an ease process avoiding hazardous chemicals, reagents, and energy.

Plant extracts contain proteins, alkaloids, terpenoids, tannins, phenols, flavonoids, sugars, and carbohydrates. These functional moieties act as a reducing and functionalizing agent in the synthesis process. Various plant part root, stem, leaves, seeds, fruits, flowers, and latex were exhaustively been used in the synthesis process (Ali et al., 2020). But until now it is very oblivious to understand the exact mechanism of plant reducing agents in the synthesis process.

Metallic nanoparticles synthesized from plants have been deployed in various applications such as antibacterial, larvicidal, nematicidal, fungicidal, dye degradation, bioleaching, bioremediation, insecticides, fertilizers, inoculants, anticancer, bio-imaging, biosensors, catalyst, and diagnostic agent (Duhan et al., 2017). The usage of these nanoparticles is rapidly applying all forms of applications due to the nontoxicity and eco-friendly principle.

In this pipeline, it is indeed to explore the potential of microbial nanoparticles; since the microbes are also an important bio-resource and its perspective in applications of nanoparticles is a needed one. Henceforth, the microbial source as a template for nanoparticle synthesis is to be used exhaustively in all forms like bacteria, fungi, algae, yeast, and actinomycetes.

4 Microbial synthesis of nanoparticles

4.1 Bacteria-mediated nanoparticles

Bacteria are a unique kind of microbes having the property of reducing metallic salt into metallic ions. Generically, bacteria can thrive at any kind of environmental condition; so that bacteria can produce self-defence mechanisms such as intracellular concentration, efflux pumps, metal ion concentration, and extracellular precipitation (Slavin et al., 2017). This inherent principle is employed for nanoparticle synthesis.

Bacteria can synthesize nanoparticles either through intracellular and extracellular pathways. The first instance of bacterial nanoparticles was reported by Beveridge and Murray (1980) synthesized gold nanoparticles from the bacteria *Bacillus subtilis*. The later variant facet of metallic nanoparticles like silver, gold, copper, iron, zinc, and palladium nanoparticles is established. Sunkar and Nachiyar (2012) reported the silver nanoparticles from *Bacillus cereus* with the spherical structure in the size 20–40 nm. Moreover, the synthesized silver nanoparticles exhibited good antagonistic activity against selected pathogens. *Stenotrophomonas malophilia* (AuRed02) mediated gold nanoparticles exhibited monodispersed gold nanoparticles with a size of 40 nm (Nangia et al., 2009). *Shewanella oneidensis* a metal-reducing bacterium is exploited for the synthesis of copper nanoparticles using a simple green chemistry approach and applied in the catalyst process (Kimber et al., 2018). Iron reducing bacteria *Actinobacter* sp. produced magnetite nanoparticles under anaerobic conditions with defined morphological features (Bharde et al., 2005).

Bacillus megaterium cell-free extract is employed as a bio-reducing agent in the formation of zinc oxide nanoparticles. The synthesized nanoparticles were in the nano regime; exhibited a multidimensional effect against *Helicobacter pylori* and assessed its efficacy in normal human mesenchymal stem cells (hMSc) (Saravanan et al., 2017). In another study, *Desulfovibrio desulfuricans* and *Escherichia coli* were successfully utilized for palladium nanoparticles (Gomez-Bolivar et al., 2019). The synthesized nanoparticles exhibited a similar degree of variation in size, shape, and other features when compared. Sulfur reducing bacteria *Desulfovibrio desulfuricans* and *Acinetobacter calcoaceticus* produced amorphous platinum nanoparticles reduced and stabilized by culture filtrate of bacteria (Martins et al., 2017; Gaidhani et al., 2014). Nickel nanoparticles were synthesized by *Proteus penneri* in a single step process method (Prabhuswamy Spoorthy et al., 2017). The synthesized nanoparticles eventually exhibited antimicrobial, antiinflammatory, and antiproliferative activity in a splendid manner. Thus, in this section, we have emphasized the microbial synthesis of metallic nanoparticles by the bacterium. All forms of metallic nanoparticles are synthesized by different genera and species of bacteria and displayed prominent activity.

4.2 Actinomycetes-mediated nanoparticles

Actinomycetes are a group of heterogenous gram-positive bacteria with fungal morphology (Barka et al., 2015). The bacteria habitat in marine sediments and conceptually protect the marine ecosystem. These kinds of microbes are highly explored for the synthesis of nanoparticles due to various factors; actinomycetes produce a wide variety of antibiotics as secondary metabolites.

Marine actinomycetes *Streptomyces rochei* MHM13 is employed for silver nanoparticles (Abd-Elnaby et al., 2016). Using extracellular culture filtrate the actinomycete strain effectively reduces silver metals into silver ions. Moreover, the synthesized nanoparticles progressively inhibited the growth of bacterial pathogens and insist toxicity in cancer cell line hepatocellular carcinoma cells (HepG-2), breast carcinoma cells (MCF-7), colon carcinoma cells (HCT-116), prostate carcinoma cells (PC-3), lung carcinoma cells (A-549), intestinal carcinoma cells (CACO), larynx carcinoma cells (HEP-2), and cervical carcinoma cells (HELA). Soil actinomycete *Streptomyces griseoruber* extracellular filtrate strategically reduced the gold chloride into gold ions; the produced gold nanoparticles were crystalline and performed catalysts activity (Ranjitha and Rai, 2017). *Streptomyces capillispiralis Ca-1* filtrate produced monodispersed copper nanoparticles with significant size, shape and displayed an antibacterial and biocontrol activity against infectious pathogens and phytopathogenic fungi (Hassan et al., 2018). Similarly, actinomycete strain VITBN4 effectively synthesized copper oxide nanoparticles and performed antagonistic activity against *Staphylococcus aureus* (MTCC 3160) *Proteus mirabilis* (MTCC 425), *Bacillus subtilis* (MTCC 1305), *Aeromonas caviae* (MTCC 7725), *Aeromonas hydrophila* (MTCC 1739), *Edwardsiella tarda* (MTCC 2400), and *Vibrio anguillarum* (Nabila and Kannabiran, 2018).

Streptomyces minutiscleroticus M10A62 isolated from magnesite mine can synthesize selenium nanoparticles from the extracellular filtrate (Ramya et al., 2015). Further, selenium nanoparticles exhibited better antibiofilm, antioxidant, wound healing, and antiviral activity. In another interesting study, zinc nanoparticles were synthesized from actinomycete strain and evaluated its potency against tested pathogens (Rajamanickam et al., 2012). Moreover, further, these nanoparticles can be utilized for antibacterial coating in food preservation.

4.3 Fungi-mediated nanoparticles

The fungus is another group of eukaryotic members which includes yeasts, fungi, and molds. The inherent ability of fungi is tolerance to metals, accumulate the metals and reduce the metals into respective ions (Ayangbenro and Babalola, 2017). Further, synthesis of nanoparticles is easy with fungi compared with bacteria owing to the extracellular synthesis of proteins by fungi; good biomass production; large scale synthesis; easy approach to culture the filtrate and optimization parameters will tune the fungi to synthesis large quantity of nanoparticles (Guilger-Casagrande and De Lima, 2019).

Trichoderma longibrachiatum extracellular culture filtrate is used as a reducing and stabilizing agent for the synthesis of silver nanoparticles (Elamawi et al., 2018). The synthesized nanoparticles are in well-defined morphology and portrayed good antagonistic activity against plant pathogenic fungi *Fusarium verticillioides, Fusarium moniliforme, Penicillium brevicompactum, Helminthosporium oryzae, and Pyricularia grisea*. Molnár et al. (2018) screened 29 thermophilic fungi for the production of gold nanoparticles with varying different conditions. Among the 29 isolates, *Rhizomucor pusillus, Sporotrichum thermophile, Termoascus thermophilus,* and *Termomyces lanuginosus* effectively produced monodispersed gold nanoparticles. *Stereum hirsutum*, a Native White-Rot Fungus can synthesize copper/copper oxide nanoparticles under different pH conditions with three different salts precursors.

Manglicolous fungi, *Fusarium incarnatum* (STSP 27), *Phialemoniopsis ocularis* (STSP 19), *Trichoderma asperellum* (STSP10), and *Penicillium pimiteouiense* (STS28) effectively reduce the ferric chloride and ferric dichloride into respective ferric ions by co-precipitation method (Mahanty et al., 2019). Further, *Penicillium pimiteouiense* mediated iron nanoparticles effectively remove chromium ions from wastewater. Culture filtrate of *Aspergillus niger* fabricate zinc oxide nanoparticles and incorporated in cotton fabrics intended for antibacterial applications (Kalpana et al., 2018). The cotton fabrics coated with zinc oxide nanoparticles inhibited the growth of *E. coli* and *S. aureus*.

Gupta and Chundawat (2019) reported the synthesis of platinum nanoparticles from the extract of *Fusarium oxysporum*. The synthesized nanoparticles were crystalline with a face-centered cubic structure with an average size of 25 nm. Besides, platinum nanoparticles suppress the growth of *E. coli* and scavenge free radicals up to 79% drastically. Aqueous extract of *Saccharomyces cerevisiae* reduces the palladium acetate salts into palladium nanoparticles with high yield (Sriramulu and Sumathi, 2018). The synthesized nanoparticles considerably degrade azo dye in the presence of UV-light. In another study, *Saccharomyces cerevisiae* extract mediated silver nanoparticles outcome a good antimicrobial activity against methicillin-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Olobayotan and Akin-Osanaiye, 2019). Yeast cells of *Magnusiomyces ingens LH-F1* extract incur $HAuCl_4$ into respective metallic gold ions in a rapid simultaneous process. By this method, the synthesized gold nanoparticles exhibited excellent catalytic activities in the reduction of nitrophenols (i.e., 4-nitrophenol, 3-nitrophenol, and 2-nitrophenol) to aminophenols (Zhang et al., 2016). Yeast *Rhodotorula mucilaginosa* produced copper nanoparticles in a low-cost method and the nanoparticles excel its efficacy in removing heavy metals in wastewater treatment (Salvadori et al., 2014). Moghaddam et al. (2017) reported that a New *Pichia kudriavzevii* Yeast Strain can synthesize zinc nanoparticles with uniform size and shape. In connection, the nanoparticles performed superior activity against clinical pathogens. Vainshtein et al. (2014) reported magneto-sensitive nanoparticles from the extract of *Saccharomyces cerevisiae* and *Cryptococcus humicola* in a simplified process for drug delivery.

4.4 Algae-mediated nanoparticles

Algae are a rich source of potent secondary metabolites. These metabolites are widely and intensively used in pharmacy, cosmetics, detergents, food packaging, feeders, drugs for various infectious diseases. Nowadays, the use of algae in nanotechnology is a time deed one for better development of a new variant of the drug delivery module. Marine brown alga *Padina pavonia* smartly transferred silver nitrate precursor into silver ions in a single pot method (Sahayaraj et al., 2012). The synthesized nanoparticles were in par with nano feature shape and size. Likewise, *Gelidiella acerosa*, marine algae conceivably synthesized gold nanoparticles with high yield (Senthilkumar et al., 2019). In connection to that, gold nanoparticles excelled in antidiabetic, antibacterial and antioxidant activity. Likewise, *Sargassum polycystum* extract stabilized and reduced copper II sulfates into copper oxide ions and dominantly suppress the growth of bacterial cells (Ramaswamy et al., 2016). El-Kassas et al. (2016) investigated the efficacy of seaweeds *Padina pavonica* (Linnaeus) Thivy and *Sargassum acinarium* (Linnaeus) Setchell 1933 capable of synthesizing magnetite nanoparticles. The seaweeds modulate efficiently the ferric chloride salt precursor into ferric ions and it enhances the removal of lead from the contaminated part. Momeni and Nabipour (2015) investigated the synthesis of palladium nanoparticles from seaweed *Sargassum bovinum* and carried out a detailed experiment on electrocatalytic behavior. The output of the study suggests that palladium nanoparticles are a good sensor in determining hydrogen peroxide in aqueous solution.

5 Mechanism of nanoparticle formation

Though the microbes effectively convert the metallic precursors into metallic ions; the underlying principle behind the synthesis process is to be addressed. Microbes adapt a stringent etiquette to combat against in reduction of metal ions, complex formation, precipitation, dissimilatory, oxidation to fight against metal toxicity (Isa Tanzil et al., 2016). In nanoparticles synthesis by microbes, certain enzymes play an important role in transporting electrons from certain donors to positive metal ions (Siddiqi et al., 2018). The production of metal nanoparticles by microbes is executed by redox reactions through intracellular/extracellular. Initially, at first instance during nanoparticle synthesis, metal ions first trapped on the surface or inside of microbial cells. Later the trapped metal ions are exclusively converted into nanoparticles with the aid of microbial enzymes (Fig. 4).

Streptomyces sp.LK3 pragmatically synthesized silver nanoparticles by the enzyme nitrate reductase (Karthik et al., 2014). In *Geobacter sulfurreducens* bacteria, the pili cell surface appendages accomplish the transfer of metal ions into nanoparticles. The pilin protein of pili is solely responsible for the transformation of nanoparticles (Cologgi et al., 2011). In another study, the importance of redox mediators (ubiquinol, NADH oroxygen/superoxide) and the role of c-type cytochromes redox proteins in nanoparticles synthesis is well established. *Shewanella oneidensis* MR 1 incurs metal-reducing machinery or Mtr pathway for transferring of electrons from quinol to the bacterial inner surface in iron oxide nanoparticles synthesis (Shi et al., 2012).

$$\textit{microbialextracts} + \textit{metalsalt} \xrightarrow[\textit{PROTEINS}]{\textit{NADHENZYMESYSTEM}} \textit{metalions} + \textit{byproducts}$$

Silver nanoparticles synthesized from *E. coli* produced polydispersed nanoparticles. The aldehyde and hemiacetal groups present in the extract acted as a reducing agent (Divya et al., 2016).

Bacillus licheniformis synthesized AgNPs by the nitrate reductase enzyme system. The enzyme along with NADH and other cofactors are required for the bioreduction

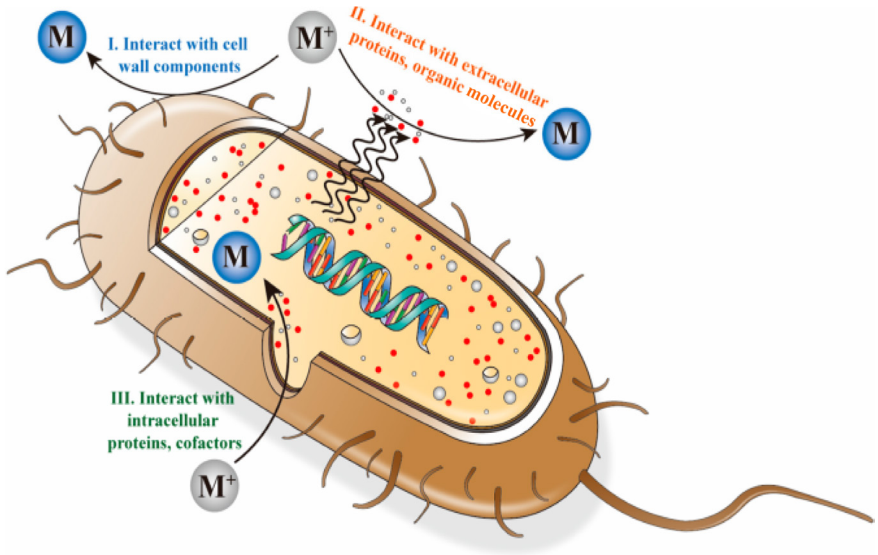

FIG. 4

Mechanism of nanoparticles synthesis by bacterial cells.

From Fang, X., Wang, Y., Wang, Z., Jiang, Z., Dong, M., 2019. Microorganism assisted synthesized nanoparticles for catalytic applications. Energies 12(1), 190, with permission from MDPI, Open Access publisher.

of Ag into Ag ions (Vaidyanathan et al., 2010). In another study, *Rhizoporous oryzae* fungi mediated gold nanoparticles suggested that the involvement of peptides or proteins of culture filtrate is involved in the reduction and stabilization of gold nanoparticles formation (Das et al., 2012).

In *Verticillium* sp., it is been postulated that synthesis of gold nanoparticles was accomplished by the electrostatic forces of attractions is required for attachment of metal salt on the surface of the mycelial cell wall due to the negative charge of the carboxylate group of enzyme; later the enzyme by the process of nucleation it reduces into metallic ions (Mukherjee et al., 2001a,b).

Colpomenia sinuosa mediated iron nanoparticles were synthesized by polysaccharides and sulfur polysaccharides present in the extract (Salem et al., 2019). The synthesis is initiated by hydrolysis of ferric chloride into $Fe(OH_3)$ and H^+ ions. Later the polysaccharides reduced the ferric hydroxide into Fe_3O_4 nanoparticles.

Microalgae *Chlorella* extract-mediated zinc oxide nanoparticles are produced at ambient conditions. The mechanism behind the synthesis is anticipated as; donor-acceptor mechanism: i.e., OH groups in carbohydrate present in chlorella extract donate an electron to electrophile zinc species leading to oxidation of hydroxyl group and reduction of electron-deficient Zn ions to Zn atoms (Khalafi et al., 2019).

Thus with the above appropriate examples herein it is been collectively concluded that chemical moieties present in the extract and with the help of microbial enzymes the reduction of metals into metal ions are accomplished.

6 Insecticidal efficacy of microbial-mediated nanoparticles

The small nano-scale materials are gaining profound importance in various kinds of environmental applications like bioremediation, bioleaching, degradation of dye, nanosensors. In this aspect, the use of nanomaterials in combating the larvae population is another notable application. As of now, variants of chemical pesticides and insecticides were aggressively utilized by governmental municipalities to knockdown the larvae breeding sources; though the treatment is effective it creates unwarranted effects on the environment like resistance, nontarget organisms and toxicity to humans also.

God blessed the nature with diversified plants and microbial sources for well-being of humans and the environment. In ancient times onwards, the use of plant oils is practiced as a larval agent to counteract the larval populations. But later, these plants and their products are intensively utilized and explored to develop some mosquitocidal agents like Pyrethrum (*Tanacetum cinerariifolium*), neem (*Azadirachta indica*), sabadilla (*Schoenocaulon officinale*), tobacco (*Nicotiana tabacum*), and ryania (*Ryania speciosa*) (Singh and Saratchandra, 2005). These botanical pesticides were ecofriendly, low toxicity and easy biodegradability.

Microbial pesticides are the practice of using whole microbes or its product to nullify the insects sustainably. For a decade, *Bacillus thuringiensis israelensis Bti* and *Bacillus sphaericus 2362* species were utilized for Insect Vector Management (IVM) (Derua et al., 2018). Likewise, in fungi, *Beauveria bassiana, Metarhizium anisopliae, Nomuraea rileyi, Paecilomyces farinosus,* and *Verticillium lecani* were developed as biopesticides (Litwin et al., 2020). In this pipeline, it is an utmost strategy to involve nano-based concepts in microbial pesticides to develop a novel benign material with high efficacy in controlling mosquito populations.

Dharmadurai and Ramasamy (2013) investigated the synthesis of silver nanoparticles from the culture filtrate of *A. bisporus, E. coil, Penicillium* sp., *Vibrio* sp., and assayed against the *Culex* larvae. The results outcome that all the culture filtrates performed 100% mortality in a shorter time.

Salunkhe et al. (2011) reported the synthesis of silver nanoparticles from filamentous fungi *Cochliobolus lunatus* and evaluated against IInd, IIIrd, IVth instar larvae of *A. aegypti* and *Anopheles stephensi* with significant LC_{50} value—1.29, 1.48, and 1.58—*A. aegypti*; 1.17, 1.30, and 1.41 for Anopheles *stephensi*. Moreover, predatory fish *Poecilia reticulate* was exposed to myogenic nanoparticles for assessment of toxicity; nanoparticles didn't exert any negative implications.

Bacillus thuringiensis is employed as a reducing agent to synthesize cobalt nanoparticles and assessed against dengue vector *Aedes aegypti* (LC_{50}—2.87 mg/L) and malaria vector *Anopheles subpictus* (LC_{50}—3.59 mg/L). The nanoparticles exert prompted activity in the mosquito vectors in a quick time (Marimuthu et al., 2013).

Listeria monocytogenes, Bacillus subtilius, and *Streptomyces anulatus* mediated silver nanoparticles effectively dropped out the survival of mosquito vectors *Culex quinquefasciatus* and *Anopheles stephensi* at different instar larvae stage from I–II–III–IV–Larvae–Pupae–Adult (Soni and Prakash, 2015).

Phomopsis liquidambaris mediated silver nanoparticles rapidly exert a toxic effect in the II and IV instar larvae of *A. aegypti* and *C. quinquefasciatus*. The nanoparticles smartly trigger the larvicidal effect in the vector within a short frame time with LC_{50} *A. aegypti*—II instar larvae—0.761 PPM; IV instar larvae—1.015 PPM; *C. quinquefasciatus*—II instar larvae—0.791 PPM; IV instar larvae—1.715 PPM (Seetharaman et al., 2018).

Same genera but different species *Pseudomonas fluorescens YPS3, Pseudomonas mandelii* two different strains isolated from different geographic regions were utilized for silver nanoparticle synthesis and evaluated against mosquito vectors *Aedes subpictus*, *C. tritaeniorhynchus, A. aegypti, A. stephensi,* and *C. quinquefasciatus*. The results were notable one each mosquito species respond to silver nanoparticles with a higher degree of affinity variation and cause mortality with the response to dose and time (Kalaimurugan et al., 2019a,b; Mageswari et al., 2015).

Fouda et al. (2020) reported endophytic *Streptomyces capillispiralis* Ca-1, *Streptomyces zaomyceticus* Oc-5, and *Streptomyces pseudogriseolus* Acv-11 mediated silver nanoparticles effectively inhibit the larvae growth in *Culex pipiens* and *Musa domestica*. In *C. pipiens* the activity is significant with the response to dose concentration with a time interval (24, 48, 72 h).

Interestingly in another study, silver and gold nanoparticles were synthesized from fungi *Chrysosporium keratinophilum* and *Verticillium lecanii* and investigated the lethal effects of silver nanoparticles in larvae and pupae of *Aedes aegypti, Culex quinquefasciatus,* and *Anopheles stephensi*. In this study, AgNPs exert better inhibitory activity than AuNPs (Soni and Prakash, 2014).

Silver nanoparticles generated from *Bacillus amyloliquefaciens* has shown larvicidal and pupicidal activity against *Culex pipiens pallens* and determined the LC_{50} values for 1st instar larvae—0.72 ppm, IInd instar larve—0.73 ppm, IIIrd instar larvae—0.69 ppm, IVth instar larvae 1.16 ppm and 4.18 ppm for Pupae (Fouad et al., 2017). Cerium oxide nanoparticles synthesized from the culture filtrate of *Aspergillus niger* were bestowed with larval growth inhibitory activity against *A. aegypti* with LC_{50} 0.250 mg/L (Gopinath et al., 2015).

From probiotic strain *Bacillus licheniformis* Dahb1, using the exopolysaccharide, zinc oxide nanoparticles were synthesized and evaluated against malaria and Zika virus vectors. The output of the study claim that zinc oxide nanoparticles synthesized from exopolysaccharide are a potent larvicidal agent causing 100% mortality against *A. aegypti* and *A. stephensi* at very low doses (Abinaya et al., 2018).

Shanmugasundaram and Balagurunathan (2015) postulated the larvicidal activity of bacteria mediated nanoparticles from actinobacterium, *Streptomyces* sp. M25. The nanoparticles rapidly act against malarial vector *Anopheles subpictus* in a dose-dependent method (LC_{50}—51.34 mg/L); dengue vector, *Aedes aegypti* (LC_{50}—60.23 mg/L); filarial vector, *Culex quinquefasciatus* (LC_{50}—48.98 mg/L).

In another report, within 1 h of exposure of silver nanoparticles to the *C. quinquefasciatus,* it exerts 100% toxicity; while the same synthesized AgNPs were less susceptible to *An. stephensi* and *Ae. aegypti*. At the same time, pupicidal effect was also examined; the result declared that was observed higher in *A. aegypti* LC_{50}—4 ppm;

C. quinquefasciatus LC_{50}—9 ppm; *An. stephensi* LC_{50}—12 ppm (Soni and Prakash, 2013).

Banu et al. (2014) isolated *Bacillus thuringenesis* isolated from rhizosphere soil and synthesized silver nanoparticles. The synthesized nanoparticles render very strong antagonistic activity against third instar larvae of *A. aegypti* with significant LC_{50} 0.10 ppm.

Ulva lactuca green alga fabricated zinc oxide nanoparticles effectively killed the fourth instar larvae of *A. aegypti* within 24 h with LC_{50}—154.67 μg/mL. Moreover, the synthesized zinc oxide nanoparticles disintegrated the internal body structures and leads to mortality (Ishwarya et al., 2018).

Soni and Prakash (2012a,b) examined the larvicidal activity of silver and gold nanoparticles synthesized from pathogenic fungi *Chrysosporium tropicum*. The result acclaims that silver nanoparticles are having better inhibitory activity than gold nanoparticles in I–II–III–IV instar larvae of *A. aegypti*.

Deepak et al. (2018) conceived seaweed *Turbinaria ornata* mediated gold nanoparticles and assessed its efficacy in female mosquito *A. stephensi*. As a result, the synthesized gold nanoparticles lucidly performed bioactivity with LC_{50} 12.79 μg/mL.

Selenium nanoparticles synthesized using *Penicillium corylophilum* displayed prompt larvicidal activity against the IIIrd instar larvae of *A. stephensi*. Moreover, pupicidal and adult emergence are confined by the action of selenium nanoparticles (Salem et al., 2020).

Amerasan et al. (2015) finely optimized and synthesized silver nanoparticles from *Metarhizium anisopliae* extract. The synthesized nanoparticles were explored as larvicides and pupicidal in I–IV instar larvae of *A. culicifacies*. The nanoparticles elevate the mosquito larva and pupa with LC_{50}—32 ppm; 39 ppm; 45 ppm; 51 ppm; pupa—60 ppm.

Kalaimurugan et al. (2019a,b) reported the synthesis of copper nanoparticles from the filtrate of *Fusarium* sp. The study was intensively conducted with mosquito vector *A. aegypti*, *C. quinquefasciatus*, and *A. stephensi*. In all the three species copper nanoparticles remarkably performed superior activity and cause 100% mortality.

Madhiyazhagan et al. (2015) formulated silver nanoparticles from *Sargassum muticum* and tested against the mosquito vectors *A. aegypti*, *C. quinquefasciatus*, and *A. stephensi* in a systemic approach as larvicidal, pupicidal, and ovicidal. In all the assays the silver nanoparticles trigger the desired effect (toxicity) in a stipulated time proportionate with dose concentration.

Sundaravadivelan and Padmanabhan (2014) interrogated the larvicidal and pupicidal assay of silver nanoparticles generated from the filtrate of *Trichoderma harzianum* in mosquito vector *A. aegypti*. The assay outcome that silver nanoparticles cause lethal effect in all the instar larvae (I–IV) and pupae also.

Banu et al. (2014) determined the acute toxicity of entomopathogenic fungi *Beauveria bassiana* mediated silver nanoparticles against the instar larvae (I–IV) of *A. aegypti*. The result is strikingly excelled with 100% mortality in all forms of larvae of *A. aegypti*.

Thus, so far we have outlooked the efficacy of metallic nanoparticles against different instar forms of larvae; from the above-mentioned report, it is evidenced that metallic nanoparticles cause rapid antagonistic activity in larvae. But the action of metallic nanoparticles against the mosquito vectors is different in general, species of mosquitoes. Also, dose and surface molecules capping the nanoparticles have a profound influence on toxicity. Some examples of metallic nanoparticles synthesized by microbes were described in Table 1.

7 Future perspectives

The synthesis of plant-mediated nanoparticles is comparatively easier than the microbial synthesis process. The drawback of microbial nanoparticles is the need to isolate, purify and extract the active components. Moreover, synthesizing nanoparticles at large scale levels is tedious for microbial nanoparticle synthesis. Further, challenges like controlling the size and shape to achieve monodispersity are bottleneck factor. The underlying mechanism behind the formation of the nanoparticles is undiscoverable. These problems should be addressed before employing these nanoparticles for commercialization.

In this chapter, we have emphasized the metallic nanoparticles synthesized by microbes and their larvicidal potential. In the context of the larvicidal activity, all metallic nanoparticles (Silver, gold, copper, iron, and zinc) executed toxic levels at variant degrees with respect to genera, species, time, dose, and surface capping agent that capped the nanoparticles. So all metallic nanoparticles can cause lethal effects in different forms of mosquito vectors, i.e., instar larvae (I–II–III–IV)–pupae–adult.

To bring out these metallic nanoparticles as successful alternative pesticides the following strategies can be followed:

- Isolation, identification of virulent strains of bacteria, actinomycetes, fungi, and yeast having insecticidal property.
- Elucidation of bioactive components from virulent strains in a simplified manner.
- Scale-up of microbial products in a controlled manner.
- Synthesis, optimization of nanoparticles from virulent strains/biomolecules in a simple step method.
- Producing monodispersed nanoparticles with uniform size and shape.
- The synthesized nanoparticles should exert sustained effect rather than a burst effect in larvae breeding sources.
- The nanoparticles can be designed to adapt in all the environmental factors (pH, water, light, and others).
- The nanoparticles should be specific to target mosquito; should not cause any lethal effect to nontarget organisms.
- Finally, the nanoparticles should not liberate or produce any byproducts which deteriorate the ecosystem.

Table 1 List of representative examples of metallic nanoparticles synthesized by microbes.

S.·no	Microbes	Name of the species	Metal nanoparticles	Size	Applications	References
1.	Bacteria	*Shewanella*	AgNPs	2–11 nm; crystalline spherical shape	Lithium ion batteries	Kim et al. (2018)
2.		*Streptomyces s. Al-Dhabi-87*	AgNPs	20–50 nm; spherical shape	Antimicrobial activity	Al-Dhabi et al. (2018)
3.		*Escherichia coli*	AgNPs	25 nm; spherical shape	Antibacterial activity	Divya et al. (2016)
4.		*Nocardiopsis dassonvillei* NCIM 5124	AuNPs	10–25 nm; spherical shape	Osteogenesis in gingival mesenchymal stem cells	Bennur et al. (2020)
5.		*Sphingobium* sp.	AgNPs	07–22 nm; spherical shape	Antibacterial activity	Akter and Huq (2020)
6.		*Streptomyces bikiniensis strain Ess_amA-1*	SeNPs	600 nm in length; 17 nm diameter	Anticancer activity	Ahmad et al. (2015)
7.	Actinomycetes	*Streptomycetes* sp. *192ANMG*	AgNPs	10 nm; spherical	Antibacterial activity	Ahmed et al. (2020)
8.		*Streptacidiphilus durhamensis*	AgNPs	68 nm; spherical	Antibacterial and antifungal	Chauhan et al. (2013)
9.	Fungi	*Penicillium oxalicum*	AgNPs	60–80 nm	Antibacterial activity	Feroze et al. (2020)
10.		*Thermphilc filamentous fungi*	AuNPs	6–40 nm	–	Molnár et al. (2018)
11.		*Aspergillus flavus*	ZnS and ZnS: Gd NPs	12–24 nm; 10–18 nm	Optical detection	Uddandarao et al. (2019)
12.		*Aspergillus welwitschiae KY766958*	Te NPs	60–80 nm/oval spherical shape	Antibacterial activity	Elsoud et al. (2018)
13.	Yeast	*Yarrowialipolytica*	AuNPs	100 nm; polygonal or spherical in shape	Cytotoxicity	Ben Tahar et al. (2019)
14.	Algae	*Chlorella ellipsoidea*	AgNPs	200 nm; spherical	Dye degradation	Borah et al. (2020)
15.		*Sargassum myriocystum*	AgNPs	20 nm; hexagonal	Environmental applications	Balaraman et al. (2020)
16.	Virus	*Squash leaf* curl *China virus* (SLCCNV)	Gold and silver	5–12—AgNPs 5–20—AuNPs	Electrical conductivity	Thangavelu et al. (2020)

Henceforth, these strategies can be followed up to develop the metallic nanoparticles as a commercial alternative for chemical pesticides.

8 Conclusion

The modern science field will always develop some novel intriguing module for the needs of human beings and the environment also. In this junction, the development of novel biopesticides from natural resources is in dire need. Thus, in this chapter, we conceptualize the importance of microbial nanoparticles with special reference to insecticidal activity. Further, we enlisted the metallic nanoparticles larvicidal activity in various forms in an elusive manner. Moreover, we strikingly advocate the perspective feature for the development of novel metallic nanoparticles with sustained release of insecticidal activity. Therefore, we conclude that microbial mediated nanoparticles are a new paradigm to combat mosquito vectors systemically.

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