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REVIEW ARTICLE

Nano-biopesticide: A novel and sustainable approach towards pest and disease management

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Abstract

This article reviews nano-biopesticides as environmentally friendly alternatives to conventional chemical pesticides in modern agriculture. Synthesis, characterization and functional properties of various nanomaterials, including biopolymer-based nanoparticles (NPs) like zein and chitosan, as well as metallic NPs, silver, gold, titanium dioxide and zinc oxide. These nanostructures are more effective against a variety of pests and diseases because of their improved bioactivity, stability and targeted delivery. Safer agricultural practices are promoted by the use of nano-biopesticides, which have several benefits such as lower pesticide dose, less environmental pollution and less damage to non-target species. The review also looks at how certain nanomaterials work, such as by interfering with pest physiology or rupturing microbial membranes, which adds to their effectiveness. Additionally, it assesses the possible harm that NP exposure may cause to ecosystems and human health, highlighting the significance of safety evaluations and regulatory frameworks. The article highlights recent developments in nanotechnology, such as green synthesis approaches, to provide environmentally sustainable pest management options. Despite their promise, challenges such as cost, scalability and long-term environmental impacts require further investigation. In order to enhance nano-biopesticide formulations, guarantee safety compliance and make it easier to incorporate them into sustainable pest control programs, the prospects section emphasizes the value of multidisciplinary collaboration. Overall, nanotechnology holds potential to revolutionize pest control by offering highly effective, safe and sustainable alternatives that support integrated pest management and agricultural sustainability.

Keywords: biopolymer nanoparticles; eco-friendly pest control; environmental safety; metal nanoparticles; nano-biopesticides; nanotechnology; pest management; sustainable agriculture

Introduction

At present, the global population has crossed 7 billion and it is expected to reach 9.2 billion by 2050, an increase of approximately 30 %. This increased population leads to a 70 % increase in food demand (1). One approach to satisfy the increased food demand is an increase in crop production and yield. While in agriculture, the reduction in crop yield and food quality for human beings is a major problem. The causes behind the crop yield losses are numerous, but the major challenge to crop yield production is the losses caused by pests and diseases that need to be managed. Therefore, it is critical to comprehend the challenges posed by pest and pesticide use. Over 65000 pests and pathogens are known to cause infestations and diseases in crop plants (2). An estimated amount of 2000 billion USD crop loss is caused each year as a result of pests and diseases. In order to reduce the severe food crises, various pesticidal technologies ought to be applied in these situations, especially in developing nations (3). Insect pest control is a major concern for entomologists, parasitologists and agricultural analysts because of the substantial and prolonged negative impacts on agricultural production and human health (4, 5). Pesticides are applied to plants to keep them healthy and manage pests and diseases. There are many chemicals used as pesticides, but the most prevalent active ingredients (AIs) in pesticides are: chlorinated hydrocarbons, organophosphates, carbamates and carbamide derivatives (6). Pesticides can be categorized as: insecticides, rodenticides, nematicides, fungicides, bactericides, herbicides and weedicides according to their mode of action, nature and structure, but these pesticides can be toxic to humans (7). These chemicals can contaminate the environment and cause harm to humans and animals by ingestion, inhalation and skin absorption (8). Moreover, the use of traditional pesticide formulations has numerous shortcomings, like dust drift, limited dispersibility, longer retention of chemical residue in soil, high organic solvent content and other related issues. Because of these restrictions, just 1 % of pesticides remain on surfaces andthe rest will be released into the environment (9). However, due to excessive use of pesticides, several pest species have become resistant to pesticides and outbreaks produced by arthropods have caused issues globally (10). Therefore, researchers are now focusing on eco-friendly Integrated Pest Management methods (11, 12).

Approximately 1 million deaths and chronic illnesses are caused by pesticide poisoning per year in the world (13). There is a need for eco-friendly and non-toxic alternatives to synthetic pesticides. Researchers and scientists have developed natural and synthetic pesticides that are less toxic, target specific, efficient even in low doses and break down faster than traditional pesticides (14). Biopesticides and nano-pesticides are introduced and applied as better alternatives to synthetic pesticides (15). The introduction and application of nanotechnology in the synthesis of pesticides has given ancient materials new properties, which is another contentious issue surrounding biopesticides (16-20). Recently, there has been a notable surge in the synthesis and utilization of nanomaterials and advancements in pesticide formulations (21, 22). Additionally, nano-carrier materials enable a regulated release and prevent active ingredients from degrading.

Recently, several compounds of natural and microbial origin have been studied as potential biopesticides, such as olive mill oil, stilbenes in grape cane, *Talaromyces flavus* strains (SAY-Y -94-01), *Clitoria ternatea*, etc. (23, 24). Emerging data indicate that nanotechnology can be a useful tool for developing new nanocomposites that manage pests and diseases and thus enhance crop varieties (25-27). Exploiting nanotechnology for the synthesis of eco-friendly pesticides leads to the emergence of nano-biopesticides. Nano-biopesticides offer several advantages over biopesticides and traditional methods alone for varied reasons, such as better and easier translocation of the compound to the plants, biodegradability, produce rapid effects after the application of the product and release from the vector gradually. They also had no negative impact on soil microorganisms; hence, they do not disturb the soil microfauna. The nano-biopesticides can be synthesised by 2 methods. In the first method, the biologically active pesticidal compound (APC) is extracted from plants then it is mixed with suitable nanoparticles (NPs). After mixing, it is inserted into a suitable supporting material that is usually a polymer. The metallic salt was secreted by the APC, which binds with NPs that hemolyze and merge into a suitable polymer; while in the second method, the biologically APC is integrated with NPs.

Biopesticides

The term "biopesticide" refers to insecticides made from microorganisms, plants and animals. These are categorized as: (a) microbial biopesticides; (b) botanical biopesticides; (c) plant-incorporated protectants; and (d) biochemical biopesticides (28). The biopesticides have many advantages over conventional pesticides, which are as follows: (i) reduced toxicity; (ii) target specific, i.e., affecting exclusively the pest in question; (iii) highly potent even at low dosages; (iv) quick breakdown; and (v) little exposure with nearly no emission problems (29).

Microbial biopesticides

In the case of diseases, the microbial biopesticide can affect the pathogen in many ways, like competition, antibiosis, induced systemic resistance, lytic enzymes, etc. In order to manage insect pests, fungus spores were initially employed as a biopesticide in the late 1800s. Agostine Bassi proved in 1835 that silkworms may be shielded from illness by the spores of *Beauveria bassiana* (white muscardine fungus). This was among the first instances of biopesticide application that were recorded. *Bacillus thuringiensis* (Bt) is a common microbial insecticide. Various Bt strains produce different combinations of proteins that kill insects or larvae (30). *Pseudomonas* and *Trichoderma* are bacterial or fungal microbes that have been widely used as biopesticides to stop the spread of soilborne diseases (31). *Trichoderma* is efficient against a varied pathogenic fungus, such as *Phytophthora, Fusarium, Sclerotinia, Sclerotiorum, Candida albicans, Rhizoctonia, Pythium*, etc. Several microbial-based biopesticide products have already been commercialized for plant-disease management, as listed in Table 1.

Botanical biopesticides

The exploitation of several secondary metabolites derived from plants, including phenolics, terpenes, alkaloids and essential oils, forms the botanical biopesticides (32). These plant extracts can act as fungicides, bactericides, insecticides and acaricides. There

Table 1. List of commercially available microbial products against plant diseases

S. No.	Microbe (Source)	Product name	Target pathogen
1.	*Agrobacterium radiobacter* strain 84	Galltrol	*A. tumefaciens*
2.	*Bacillus subtilis* strain GB03	Companion, Kodiak	*Fusarium, Rhizoctonia*
3.	*Pseudomonas syringae*	Bio-save 10LP	*Botrytis, Mucor, Penicillium*
4.	*Burkholderia cepacian*	Intercept	*Pythium, Fusarium, Rhizoctonia solani*
5.	*Pseudomonas fluorescens* A506	BlightBan A506	*Erwinia amylovora*
6.	*Pseudomonas fluorescens*	Dagger G	*Pythium, Rhizoctonia*
7.	*Aspergillus niger* AN-27	Kalisena	*Rhizoctonia solani*
8.	*Trichoderma harzianum*	RootShild, F-stop, Trichodex	*Pythium, Fusarium, Rhizoctonia, Colletotrichum, Sclerotinia*
9.	*Gliocladium virens* GL-21	SoilGard	*Pythium, Rhizoctonia*
10.	Combination of *T. harzianum* and *T. viride*	Trichopel, Trichojet	*Armillaria, Fusarium Phytophthora, Pythium*

are various plants whose extracts have been recognized as the source of agricultural pesticides and protect plants from unwanted diseases and pest attacks. Some plant extracts can also show nematicidal properties. When the juvenile root-knot nematode is exposed to *Nerium oleander's* extract at a 5 % concentration, the mortality rate of the juvenile root-knot nematode is increased.

Plant-incorporated protectants (PIPs)

These are biopesticides obtained from plant products. PIPs are basically genetically modified products that produce a specific pesticidal compound in a plant. These are directly expressed in plant tissue (genetically modified crop). The Bt genes integrated into plants responsible for producing substances harmful to insects, are the most thoroughly researched example of PIPs. Plant creates their own gene-specific proteins that function as poisons for pests when their genes are expressed.

Biochemical biopesticides

Natural chemicals or metabolites obtained from living organisms (plants, animals and insects) are known as non-toxic biochemical pesticides. They are used to control pests without causing their death. By acting as plant growth regulators (PGR) and attracting or repelling pests (pheromones), these compounds may aid in growth and development. Farmers employ pheromones and semio-chemicals to entice insects into a trap and manage the insect population by removing them from the field. Thus, insect pheromones can be used as management and monitoring instruments against crop pests. Several direct pest management methods based on pheromones include: push-pull, attract and kill, mass trapping and mating disruption (33, 34). Biochemical pesticides mechanism of action work indirectly. It harms and kills their target indirectly. For instance, they interfere with their targets' sexual function but do not harm the target directly. Terpenoids, alkaloids, phenolics and other secondary compounds that are naturally generated from plants can be employed as biopesticides. Certain vegetable oils, including canola oil, have also been found to have pesticidal qualities (35).

Nanotechnology and nanoparticles

Biopesticides are environmentally safe, do not harm humans and animals andare a better option than traditional chemical pesticides. But even the use of biopesticides has certain limitations that can be overcome using more advanced nanotechnology. Application of nanotechnology has a great future in the agricultural sector by improving abiotic stress-control methods and mitigating the effects of climate change (36). The application of NPs to combat abiotic stress is the emerging field of nanobiotechnology (37, 38). Nowadays, due to environmental concerns and human safety, scientists have proposed the concept of green NPs that can be economically produced by plants (39, 40). Nanosizing the pesticides will significantly enhance solubility, permeability, dispersion, penetration and bioactivity of the pesticide (41).

NPs are found in 3 size systems: monomeric, oligomeric, or polymeric particulate systems with a particular size scale (42). NPs of silver (Ag), copper (Cu), silicon dioxide (SiO_2) and zinc oxide (ZnO) are best suited for the development of biopesticides because of their important pest control qualities and ability to reduce pollution of water, soil and the environment (43). NPs have been used in agriculture to improve soil nutrients, suppress weeds, boost soil fertility and protect plants. Additionally, they serve as fertilizers and aid in the production of foliar tissue or species-specific insect repellents. For instance, Zn NPs are created by coupling the NPs with Zn metals using biodegradable chemicals. The resulting products are environmentally safe and are used to increase soil fertility. Soils treated with Zn NP have 3 benefits: they boost soil Zn levels, manage broad-spectrum agricultural pests and make vegetable crops a valuable source of zn. Similarly, NPs can be used as magic bullets to target specific plant components for pest management by combining fungicides, herbicides, pesticides, or genes (44). NPs are synthesized by physical, chemical and biological techniques based on their chemical makeup.

Categories of NPs

NPs are primarily divided into 3 groups based on their composition. These fall into the following categories: inorganic, organic and carbon-based (45).

Organic NPs

These NPs are made of organic materials like polymers, lipids, proteins, carbohydrates, etc. (46). Dendrimers, liposomes, micelles and protein complexes like ferritin are a few well-known examples of organic NPs. Organic NPs are more brittle by nature, vulnerable to temperature and electromagnetic radiation (47). They are usually biodegradable and non-toxic (48).

Carbon-based NPs

NPs in this category are made up of carbon atoms. This category includes fullerenes, carbon black NPs and carbon quantum dots. Applications for carbon-based NPs are numerous, including bioimaging (49), energy storage (50) and drug delivery (51). High strength and electron affinity are two of the special qualities of carbon-based NPs (52, 53).

Inorganic NPs

Inorganic NPs are defined as those that are not synthesized from biological or carbon-based components. The most prevalent types of NPs in this class include metal NPs, ceramic NPs and semiconductor NPs. Metal NPs can be monometallic, bimetallic, or polymetallic (54, 55). A few metal NPs have unique thermal, magnetic and biological properties. They consequently become more important building blocks for the development of nanodevices with a variety of applications (56, 57). In today's cutting-edge balanced synthesis of metal NPs in terms of size, shape and facet is necessary (58). Semiconductor materials have both metallic and non-metallic qualities and are utilized to generate semiconductor NPs. These NPs are essential parts of electrical, optical and photocatalysis devices (59, 60). Ceramic NPs are inorganic solids composed of carbides, phosphates, carbonates metal and metalloid oxides, including calcium (Ca) and titanium (Ti) (61). They are frequently created by heating and then cooling and they can be thick, porous, hollow, amorphous, or polycrystalline. An overview of commonly studied nano-biopesticides, their synthesis approaches, target organisms and pesticidal mechanisms is summarized in Table 2. They are widely used in biological applications due to their high load capacity and good stability (62). Different categories of NPs used in nano-biopesticide formulations, along with their composition, synthesis methods and agricultural applications, are summarized in Table 3.

Table 2. Summary of various nano-biopesticides, their synthesis methods, target pests and mode of action

S. No.	Type of nano-biopesticide / nanoparticle	Source/synthesis method	Active ingredient/material	Target pest or pathogen	Mode of action	Advantages/key outcomes	References
1	Silver nanoparticles (AgNPs)	Green synthesis using *Ficus benghalensis* and *Ficus religiosa* leaf extracts	AgNPs	*Helicoverpa armigera*, *Spodoptera litura*, *Aedes aegypti*	Induces oxidative stress and gut damage in larvae	High mortality rate, eco-friendly, non-resistant	(63)
2	Zinc oxide nanoparticles (ZnO NPs)	Green synthesis using plant extracts	ZnO nanocomposites	*Aedes aegypti* larvae	Disruption of midgut cells and enzyme inhibition	Biodegradable, cost-effective, low toxicity	(64)
3	Silica nanoparticles (SiO_2 NPs)	Chemical precipitation/Sol-Gel method	Silica (SiO_2)	*Plutella xylostella*, *Sitophilus oryzae*	Desiccation, cuticle damage, spiracle blockage	Physical mode of action, minimal chemical residues	(65)
4	Alumina nanoparticles (Al_2O_3 NPs)	Physical synthesis (thermal decomposition)	alumina	*Sitophilus oryzae*, *Bombus terrestris*	Cuticle degradation, dehydration	Long residual effect, non-systemic action	(66)
5	Titania nanoparticles (TiO_2 NPs)	Sol-Gel or green synthesis	TiO_2	*Drosophila melanogaster*, *Bombyx mori*	ROS generation, interference with reproduction	Stable under light, broad spectrum	(67)
6	Gold nanoparticles (AuNPs)	Green synthesis using *Cassia fistula* and *Artemisia vulgaris* extracts	AuNPs	*Aedes aegypti*, *Blattella germanica*, *Planococcus citri*	Enzyme inhibition (trypsin), cell deformation	Target-specific, high efficacy in low doses	(68)
7	Biopolymer-based nanoparticles	Encapsulation in chitosan, zein, alginate, PEG	Bioactive phytochemicals or microbial metabolites	Fungal and insect pests	Controlled release and protection of actives	Improved stability and biodegradability	(69)
8	Microbial nano-formulations	Bt–based nano-biopesticides	Bt-AgNPs, Bt-ZnO composites	*Callosobruchus maculatus*, *Aedes aegypti*	Combination of microbial toxins and nanotoxicity	Enhanced persistence and field stability	(70)

Table 3. Categories of nanoparticles used in nano-biopesticide formulations and their key applications

Category of nanoparticle	Composition/material type	Examples	Synthesis approach	Applications in agriculture/pest management	References
Organic nanoparticles	Polymers, lipids, proteins, carbohydrates	Dendrimers, liposomes, micelles, ferritin NPs	Polymeric encapsulation / emulsification	Controlled release of biopesticides, biocompatible carriers	(71)
Carbon-based nanoparticles	Carbon allotropes	Fullerenes, carbon nanotubes, graphene oxide, carbon quantum dots	Chemical vapor deposition, hydrothermal synthesis	Bioimaging, biosensing anddelivery of pesticidal compounds	(72)
Inorganic nanoparticles (metal -based)	Metals or metal oxides	Ag, Cu, ZnO, TiO_2, Au, Fe_3O_4	Chemical reduction, Sol–Gel, green synthesis	Antimicrobial and insecticidal activity; enhanced pesticide stability	(73)
Inorganic nanoparticles (ceramic-based)	Metal and metalloid oxides, carbides, phosphates	CaO, TiO_2, Al_2O_3, SiO_2	Calcination, precipitation, Sol–Gel	Long-lasting protective coatings, pest deterrence and soil stabilization	(74)
Semiconductor nanoparticles	Metallic + non-metallic elements	CdS, ZnS, TiO_2, Si	Chemical precipitation, sol–gel	Photocatalytic degradation of pesticide residues, light-activated pest control	(75)
Hybrid nanoparticles	Combination of organic + inorganic	Polymer–metal composites, nanoclays	Co-precipitation, hybrid encapsulation	Improved stability, multifunctional pesticide delivery	(76)

Nano-biopesticide

Biologically generated and designed substances with NPs used as pesticides are known as nano-biopesticides. Nano-biopesticides have intrinsic qualities including target-specific, release of active ingredients, enhanced solubility of active ingredients and the active component will not degrade too quickly and easily (77). Even when they are applied in small amounts, nano-biopesticides will not lose their effectiveness. The use of nano-biopesticides in disease and pest management can enhance the target specificity by employing information on the disease and/or pest's life cycle and behaviour. The activity of gut protease in *Helicoverpa armigera* is altered by the AgNPs synthesized from *Ficus benghalensis* (banyan tree) and *Ficus religiosa* (peepal tree) leaf extracts (78). Direct application of chemical pesticides to plants can result in environmental problems and chemicals released into the food chain through the air or soil. Pesticides containing NP formulations, such as micelles and nano-composite materials, help control these problems by lowering the likelihood of environmental and health problems. Likewise, insecticides are delivered by clay-based nanotubes to manage pests (79). Further, the addition of biopolymers to nano-biopesticides enhances numerous properties of nano-biopesticides, like stiffness, penetrability, solubility, biodegradability, thermal stability and crystallinity (80, 81). Reported nano-biopesticides exhibiting significant efficacy against diverse pest species across different countries are summarized in Table 4. The mode of action of nano-biopesticides involves inducing oxidative stress, damaging gut tissues and disrupting cellular processes in pests (Fig. 1)

Silver NPs-based nano-biopesticides

AgNPs nanoparticles have ovicidal, larvicidal, adulticidal and oviposition deterrent properties (92). They do not modify the gene expression of insects (93); thus, AgNPs are eligible to be referred to as nano-biopesticides. Biopolymers and phytoextracts are utilized in the synthesis of AgNPs. A great mortality rate was observed against *Musca domestica* by AgNPs obtained from a lemon extract base. Also, significant pesticidal activity was demonstrated against lepidopterans, aphids, thrips, beetles, moths and cotton bollworms by AgNPs. AgNPs with notable bio-pesticidal capability were produced employing natural compounds as reducing agents (94). Since NPs induce oxidative stress in the larvae's gut, (95) documented the effects of AgNPs on castor semilooper (*Acheae ajanata*) and Asian armyworm (*Spodoptera litura*). *Drosophila melanogaster* was exposed to AgNPs and it experienced increased mortality as well as issues linked to growth and development. According to investigations by several researchers, green synthesised NPs decreased the protein levels, acetylcholine and other carboxylesterase activities, which resulted in mortality in a variety of insect species (96). According to a recent study, *Aedes albopictus* larvae exposure to salicylic acid-synthesized AgNPs resulted in a drop in total protein content and decreased activity

Table 4. List of Nano-biopesticides with their efficacy, dose and country of study

Nano-biopesticide type	Source/composition	Target pest/pathogen	Dose/concentration	Efficacy (%)	Country	References
Silver nanoparticles (Ag NPs)	*Ficus benghalensis* leaf	*Aedes aegypti* larvae	25 mg/L	100	India	(82)
Zinc oxide nanoparticles (ZnO NPs)	*Azadirachta indica* leaf extract	*Helicoverpa armigera*	50 mg/L	95	India	(83)
Gold nanoparticles (Au NPs)	*Cassia fistula* extract	*Spodoptera litura*	40 mg/L	90	Sri Lanka	(84)
Silica nanoparticles (SiO_2 NPs)	Chemical precipitation	*Sitophilus oryzae*	0.1 g/kg grain	85	Belgium	(85)
Copper nanoparticles (CuNPs)	*Calotropis gigantea*	*Aphis gossypii*	60 µg/mL	92	Egypt	(86)
Titania nanoparticles (TiO_2 NPs)	Sol–Gel method	*Drosophila melanogaster*	100 µg/mL	88	China	(87)
Alumina nanoparticles (Al_2O_3 NPs)	Thermal decomposition	*Plutella xylostella*	0.5 g/L	80	Japan	(88)
Chitosan-based nanoformulation	Chitosan + neem extract	*Callosobruchus maculatus*	2 % solution	98	India	(89)
Microbial nano-biopesticide (Bt–Ag NP)	*Bacillus thuringiensis* coated with AgNPs	*Aedes aegypti*	10 ppm	100	Malaysia	(90)
Iron oxide nanoparticles (Fe_3O_4 NPs)	*Eucalyptus globulus* extract	*Tetranychus urticae*	80 mg/L	90	Iran	(91)

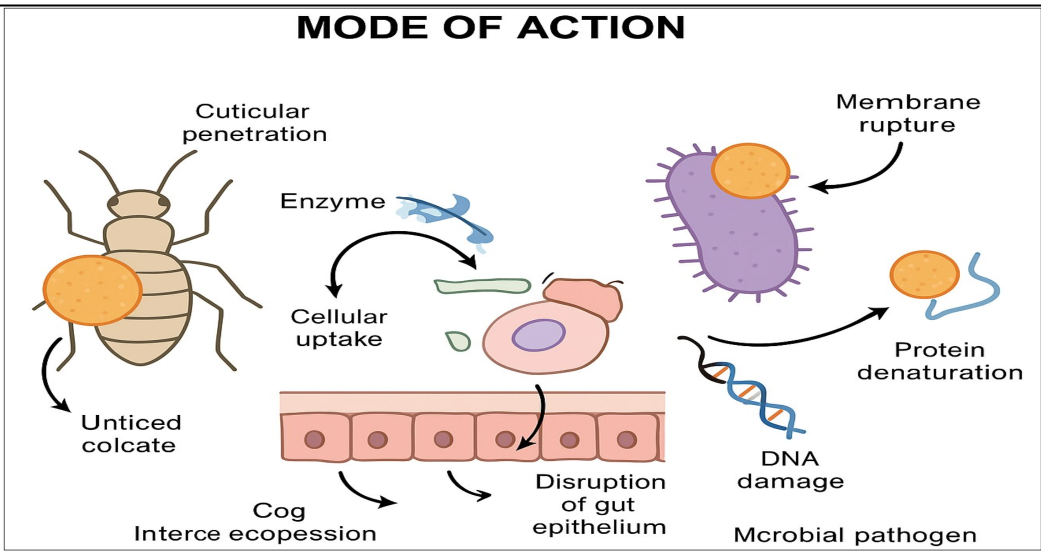

Fig. 1. Mode of action of nano-biopesticides.

of esterases and phosphatases (97, 98). Thus, in the above-described studies, exposure to green synthesised AgNPs produces considerable mortality in a variety of insect pest species, with different values of LC_{50} depending on the species, suggesting that the AgNPs have pesticidal potential (99).

Zinc oxide NPs-based nano-biopesticides

Third-instar *Aedes aegypti* exposed to ZnO nanocomposites showed significant morphological and histology-related abnormalities, including deformities of the abdomen and thorax, midgut degradation and gill loss (LC_{50}= 1.57 mg/L for 1 day) (100).

Silica NPs-based nano-biopesticides

Regarding how silica NPs work against various pests, there are a variety of physical harms (101, 102). Silica-treated NPs caused 85 % of diamondback moths (*Plitella xylostella*) to die within 72 hr as a result of spiracle blockage, desiccation and body wall breaking (103).

Alumina NPs-based nano-biopesticides

Significant pesticidal effects of alumina NPs against *Sitophilus oryzae* (rice weevil/pest) (104). The mode of action shows that cuticle degradation caused by alumina NPs results in dehydration. According to the previous study (105), Bumblebees (*Bombus terrestris*) treated with silica NPs (34 mg/L) experienced severe abnormalities in their midgut epithelium.

Titania NPs-based nano-biopesticides

Titania NPs exhibit toxic effects on *Drosophila melanogaster,* leading to reduced offspring viability (106, 107).

Gold NPs-based nano-biopesticides

By hindering the development and reproduction in German cockroaches (*Blatella germanica*), gold NPs kill the insect (108). Green synthesised gold nanoparticles (AuNPs) inhibited trypsin in mealybugs (*Planococcus citri*), mosquitoes (*Aedes aegypti)* and beetles (*Stegobium panniceum*) (109). Substantial mortality in *Aedes aegypti* was observed when exposed to AuNPs (110, 111). Exposure to AuNPs caused the midgut, cortex, caeca and epithelial cells to be mutilated andthe caudal hairs to be lost.

Synthesis of metallic NPs (nano-biopesticides)

Chemical method/bottom-up method

Bottom-up method involves sono-decomposition and reduction processes (112, 113). Chemical reduction, precipitation and the Sol-Gel synthesis technique are among the various chemical methods employed in this approach. More accurate NPs in terms of size, shape and molecular makeup are produced using this technique. Fig. 2 showed schematic representation of the green synthesis process and mechanism of metallic NPs. The various techniques used in the chemical method are as follows:

Chemical reduction

In the chemical reduction technique, NPs are created by chemically reducing the metal salts. This process is frequently used to create metallic NPs, including Au and Ag. Polyol and citrate reduction are popular approaches.

Precipitation

Using precipitation techniques, metal ions are precipitated with the addition of a precipitating agent to create NPs. This technique works well with a variety of metal NPs.

Sol-Gel synthesis

A sol, a colloidal suspension of NPs, is converted into a gel using this sol-gel synthesis technique, which is then dried and annealed to create NPs.

Physical method/ Top-down method

The Physical or top-down strategy involves grinding of bulk metallic components and stabilizing nanocomposites with colloidal protective chemicals. Despite being widely used, this process can be costly, time-consuming and result in NPs with flawed surface topologies. The various physical techniques are as follows:

Arc discharge, laser ablation and evaporation-condensation

In these techniques, a target substance is physically vaporised using arc discharge or laser ablation, resulting in the synthesis of NPs. They are frequently employed to synthesize NPs based on carbon.

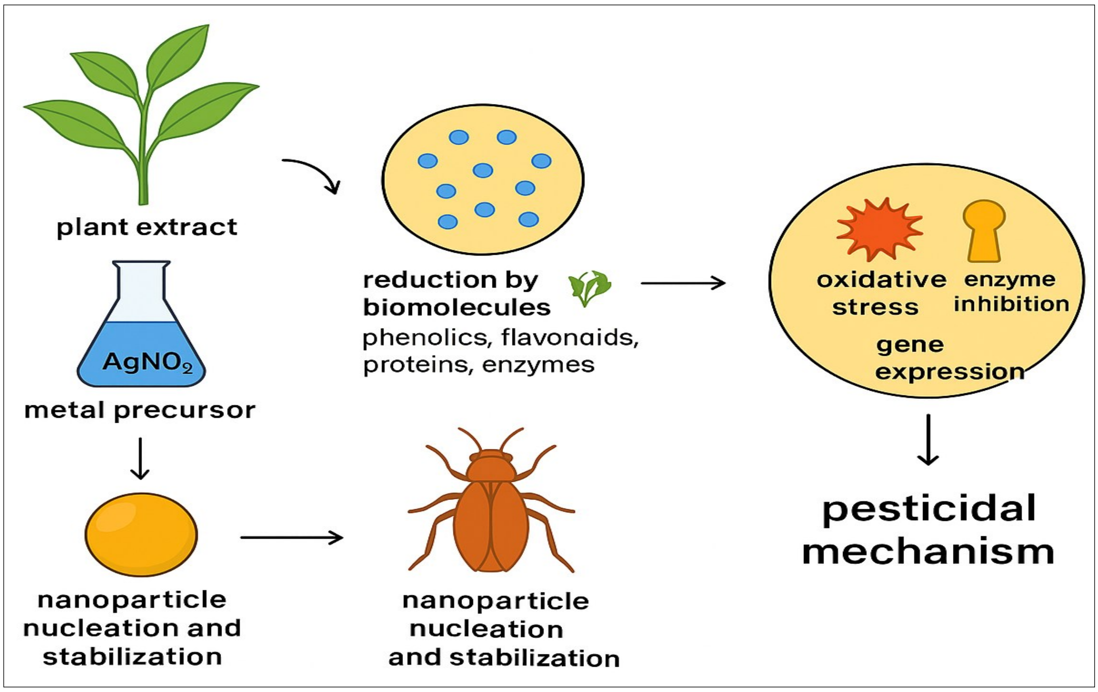

Fig. 2. Schematic representation of the green synthesis process and mechanism of metallic nanoparticles.

Ball milling

To synthesize NPs, bulk materials are ground and milled using mechanical techniques like ball milling. Metals and ceramics are among the materials that can be worked with using this approach.

Biological synthesis (green synthesis methods)

In this method, the NPs synthesis often involves the utilization of various synthetic compounds, such as sodium citrate, hydrazine hydrate and SDS (114-116). These organic reducing agents are costly, poisonous and require more energy to create NPs. They raise the biological concerns and have challenging waste management issues (117, 118). These detrimental effects have made it challenging for researchers to identify eco-friendly and natural (biopolymers) reducing agents under this method of NPs synthesis (119-122). Two biological sources are typically utilized for NP synthesis.

Microbial synthesis using different fungal/bacterial strains

This method involves using microorganisms like fungi and bacteria to biosynthesize NPs. Microbes' capacity to lower metal ions and regulate the size and structure of NPs makes this technique beneficial. Metal salt can be significantly reduced by a variety of bacterial strains, such as *Pseudomonas asstutzeri*, *Bacillus lichiniformis* and *Lactobacillus* spp. (123, 124).

Plant-mediated synthesis

The synthesis of various nanocomposite materials also involves plants that contain secondary metabolites such as flavones, amides, cyperoquinones, benzoquinones, terpenoids and ketones (125, 126).

Application strategies: Formulation and delivery of nano-biopesticides

Formation plays a crucial role in the successful application of nano-biopesticides in agriculture. A proper formulation of nano-biopesticides will enhance their stability and efficacy. Effective dispersion is a prerequisite for any nano-effective formulation in practical applications. Nano-biopesticide delivery methods with various functions for plant protection have recently been described as including nano-emulsions, nano-encapsulates, nanocontainers and nanocages. The following formulations are used for the delivery of nano-biopesticides

Encapsulation in NPs

In encapsulation, the biodegradable matrix/polymer makes the outer vesicle that encloses the active compounds in the inner core. The substance that is encapsulated in a polymeric NP is a bioactive substance. It enhances the stability and protects against environmental deterioration. By enabling regulated release, these NPs guarantee a consistent and efficient distribution of active substances.

Nano-emulsions

Nano-emulsions enhance many factors of nano-biopesticides, i.e., chemical stability, hydrophilicity, environmental durability, effectiveness of nano-biopesticides and are largely dependent on nano-emulsions. The bioavailability of active substances is improved by the small size of the droplet, which increases the surface area available for interactions. Additionally, this formulation approach ensures effective pest control by improving coverage on plant surfaces.

Nano-capsules

Nano-capsules are composed of a membrane-like structure or shell that encloses the active compound in its core. By encasing bioactive substances in a protective shell, environmental deterioration and off-target effects are reduced and stability and targeted administration are guaranteed. Long-lasting effectiveness is facilitated by the regulated release mechanisms provided by nano-capsules. Polymeric nano-capsules are used as nanocarriers, primarily composed of biodegradable polymers such as chitosan, alginate, gelatin, collagen, carboxymethylcellulose and polyethylene glycol (PEG).

Hybrid formulations

In hybrid formulations, combining various nanomaterial types can improve stability and effectiveness in a synergistic way. For multifunctionality and enhanced overall effectiveness, hybrid nano-biopesticides can contain NPs, nanocomposites, or a mix of the 2.

Co-formulants for stability

Nano-biopesticides can be made more stable by adding co-formulants or stabilizing agents. Surfactants, polymers and other stabilising agents are examples of these additives, which enhance dispersion, stop agglomeration and shield the active ingredients from deterioration.

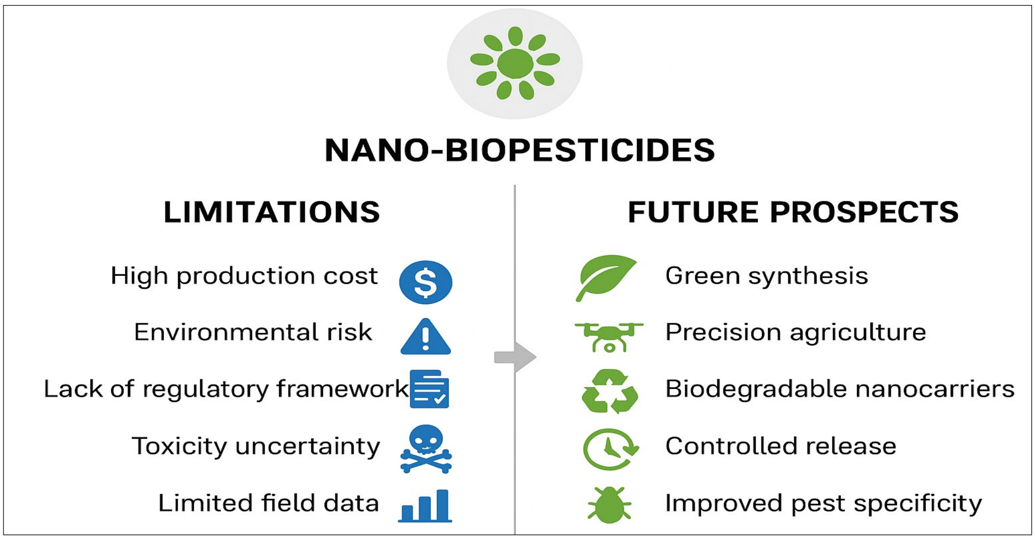

Fig. 3. Schematic representation of the key limitations and future prospects of nano-biopesticides.

Future prospects

As shown in Fig. 3 the schematic illustrates the key limitations faced by nano-biopesticides and highlights potential future prospects to address these challenges.

- Nano-biopesticides have significant potential as sustainable alternatives to conventional pesticides, but their wider adoption requires collaborative and regulatory advancements.
- Multidisciplinary collaboration among material scientists, agronomists, toxicologists, microbiologists and regulatory experts is essential to ensure safety, scalability and field efficiency.
- Standardization of ecotoxicity testing protocols is crucial, including soil microbial toxicity, aquatic organism tests e.g., zebrafish (*Daphnia magna*) and non-target species assessments such as pollinators (*Apis mellifera*) and beneficial insects (*Trichogramma spp*.).
- The development of green synthesis methods and biodegradable nanocarriers should be prioritized to minimize environmental impact and enhance biodegradability.
- Integration of nano-biopesticides with precision agriculture technologies such as drone-assisted spraying and smart delivery systems can improve targeting and reduce waste.
- Strengthened regulatory frameworks and clear biosafety guidelines are necessary for commercialization and large-scale implementation.
- Farmer training, stakeholder engagement and global research collaboration will play vital roles in ensuring the responsible use, monitoring and long-term sustainability of nano-biopesticides.

Conclusion

Nano-biopesticides represent a transformative advancement in sustainable pest management, offering enhanced efficacy, target specificity and reduced environmental impact compared to conventional pesticides. Beyond their current achievements, future progress depends on strengthening multidisciplinary collaboration among scientists, toxicologists and regulatory authorities to ensure the safe and efficient application of these technologies. Emphasis should be placed on developing green synthesis methods, biodegradable nanocarriers and standardized ecotoxicity testing to safeguard ecosystems. Integrating nano-biopesticides with precision agriculture and farmer training programs will further enhance their effectiveness at the field level. With continued innovation and harmonized regulations, nano-biopesticides have the potential to revolutionize global agriculture through sustainable, eco-friendly pest control solutions.

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Authors' contributions

JS contributed to conceptualization, literature collection and drafting of the manuscript. AK was responsible for conceptualization, supervision and critical revision of the manuscript. ND handled data curation, drafting and editing. KKY contributed to the literature survey and manuscript writing. RK prepared figures and tables and carried out literature analysis. S assisted in literature review and manuscript editing. SS contributed to data collection and manuscript drafting. SG managed literature compilation and reference organization. SK[1] provided technical inputs and revised the manuscript. AB performed proofreading and provided critical comments on the draft. PN contributed to the literature review, formatting and editing. SK[2] assisted in manuscript editing, grammar checking and final corrections. All authors read and approved the final version of the manuscript. [SK[1] stands for Sunil Kumar and SK[2] stands for Sonali Kokale]

Compliance with ethical standards

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