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Emerging Role of Entomopathogenic Fungi in Nanoparticle Synthesis and their Application in Pest Control

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Abstract

Nanotechnology research is a cutting-edge technology with several applications. One of the most difficult problems in nanotechnology is the production of nanoparticles with high monodispersity, particular composition, and size. Given this, the green manufacture of the nanoparticle is critical due to its lower toxicity to higher animals. Fungi are a better biogenic agent than other biological systems utilised for synthesis due to their diversity and better growth control. As a result, nanotechnology has been investigated for the environmentally friendly synthesis of nanoparticles from various entomopathogenic fungi and their metabolites against insect pests. Because of their great host specificity, little influence on non-target organisms, and ease of mass production, entomopathogenic fungi are utilised as excellent biopesticides. It is vital to understand the biology and mode of action of these fungi before employing them as a biocontrol agent. During the creation of metal nanoparticles by fungi, mycelium is exposed to a metal salt solution. By the catalytic effect of the extracellular enzyme and fungal metabolites, harmful metal ions are converted to non-toxic metallic solid nanoparticles in this process. Because of their physical and chemical properties, nanoparticles may be more harmful to organisms than ion forms. Nanoparticles of silver and graphene oxide have a considerable impact on insect antioxidant and detoxifying enzymes, causing oxidative stress and cell death. Polystyrene nanoparticles inhibited CYP450 iso-enzymes, while Ag nanoparticles lowered acetylcholinesterase activity. It is suggested that entomopathogenic fungus-synthesized nanoparticles would be appropriate for an environmentally safer and greener approach for new leeway in insect pest control strategy through a biological process; therefore, additional research is necessary to determine the mass production, formulation, field application, and commercialization of these nanoparticles as biopesticides.

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Introduction

Entomopathogenic Fungi (EPF) are microorganisms that are eukaryotic, filamentous, heterotrophic, and saprophytic. These fungi are required for the equilibrium of the environment's organic matter decomposition cycle, and they are pathogenic to insects but harmless to humans. Entomopathogenic fungi are used in biological pest control because they cause infection in all stages of insect development and have a variety of impacts on the host. Their route of action is linked to extracellular enzymes produced during infection and development in the insect's host body, which has a direct effect on the insect's capacity to absorb nutrients and its pathogenicity and virulence. *Beauveria bassiana, Metarhizium anisopliae*, and *Isaria fumosorosea* are extensively used in biological control programmes and the development of mycoinsecticides due to their ease of handling, understanding of their metabolism, and interaction with insects of numerous orders. When exposed to high temperatures, UV radiation, and humidity, entomopathogenic fungi have a short shelf life and low vigour, making their application in pest management challenging. So, there is a need for the development of new technologies in order to boost field efficiency and durability.

Nanotechnology, an interdisciplinary science, encompasses a wide range of physics, chemistry, and biology-related research and technologies. In 1974, Norio Taniguchi of Tokyo Science University created the word nanotechnology to characterise semiconductor techniques using nanometer-level control, such as thin-film deposition. He added, "Nanotechnology consists essentially of the separation, consolidation, and deformation of materials by a single atom or molecule." Nanotechnology is gaining pace as a viable field of study for reaching these goals, with unique techniques to generating active components with nanoscale dimensions. This extensive nanoparticle-based research is anticipated to address the primary limitations of existing pest control strategies and provide new advanced nano-based formulations that penetrate the target organism (insect), resist pest defence, are safe for plants and mammals, are cost-effective to formulate and manufacture, and ideally have a new mode of action [1].

Main body

Nanoparticles for pest control

Nanoparticles (NPs) are an ultrafine particle with dimensions between 1 and 100 nanometers and characteristics not shared by non-nano size particles of the same chemical composition [2]. The 100-nm limit is based on the idea that distinguishing characteristics between particles and bulk material typically appear at a critical size smaller than 100 nm. Due to the occasional consideration of other phenomena that increase the upper limit (such as transparency or turbidity, ultrafiltration, and stable dispersion), the prefix "nano" ("ma9mo" in Greek) is acknowledged for dimensions less than 500 nm. The size, shape (spherical, rods, tubes, irregular), surface-to-volume ratio, crystal phase (crystalline, amorphous), and chemical composition (e.g. metallic, carbon, inorganic, organic, polymeric) of these materials are crucial parameters that define many outstanding properties relevant to their application as pesticides, such as toxicity.

Mode of action of Nano formulations on insects:

There are several effects identified in insects caused by nanoparticles which severely affect insect growth and development and even sometimes cause death **(Figure 1 and Table: 1)**

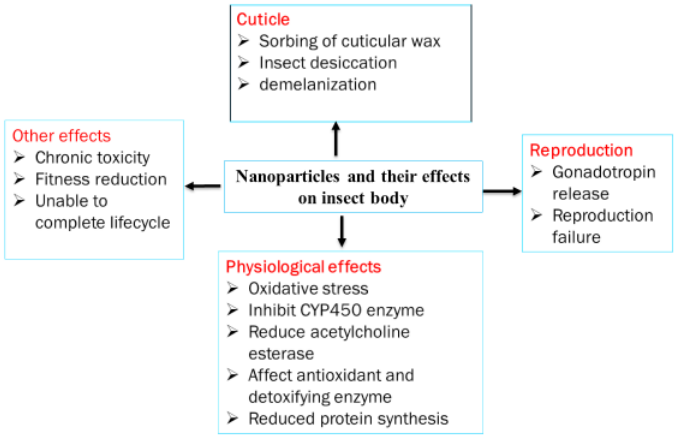

Figure 1: Effect caused by nanoparticles on insects.

i. External damage

Insecticidal dust is commonly used to protect stored grain from insects [3]. Instead of biological activity, the process in Nanostructured Alumina (NSA) dust is based on physical disruption. By sticking to the insect's cuticle via triboelectric interactions and sorbing its wax layer, charged NSA particles dehydrate the insect [4]. In addition to their hydrophobic property, these abrasive particles cause cuts and scrapes on the insect bodies [5,6]. 2015 discovered that parameters such as particle morphology, surface area, and particle size must be considered when applying insecticidal dust to maximise its efficiency. Furthermore, (E)-anethole, a significant component of *Pimpinella anisum* (aniseed) essential oil, possesses insecticidal effects. When *P. anisum* essential oil was created as a nanoemulsion against *Tribolium castaneum*, the cuticle exhibited significant damage in the form of colour alterations, muscle breakdown, thickness, and necrosis in the epidermis; cellular debris; and loss of the difference between the endocuticle and exocuticle [7]. In *Aedes aegypti* larvae, silver nanoparticles (AgNPs) produced from Pedalium murex seed extract caused hair loss on the antenna, head, upper abdomen, and lateral abdomen, as well as cuticular damage [8]. Using carbon–silver nanohybrid, [9] observed that the heads and guts of *Culex quinquefasciatus* and *Anopheles stephensi* turned black, as well as damage to the organisation of cells and cuticle membrane. This may have been caused by binding between the nanohybrid and phosphorus and/or sulphur in biological structures such as DNA and proteins. Moreover, AgNPs have been associated with depigmentation of the cuticle [10].

ii. Biochemical interactions and mobility

In addition to inflicting physical injury through contact, NPs can alter biochemical activity after being ingested or inhaled and can therefore go to the brain after inhalation [11]. Increased cytokines, Reactive Oxygen Species (ROS), and pro-inflammatory mediators, as well as alterations in membrane potential and mitochondrial respiratory chain have been associated to AgNPs [12]. Ingestion of AgNPs during early larval stages has been linked to a decrease in the capacity to crawl and climb in later larval and adult stages. Flies exposed to non-lethal amounts of AgNPs exhibited cuticular depigmentation resembling copper deficit and locomotor impairment. The activity of two copper-dependent enzymes, Cu–Zn Superoxide Dismutase (SOD) and tyrosinase, was shown to be diminished. These enzymes are respectively engaged in antioxidant action and pigment production [10]. Calexcitin is a protein responsible for binding calcium and causing membrane excitability. AgNPs

have been demonstrated to inhibit Calexcitin [13]. In research on honey bee brains using zinc oxide nanomaterials (ZnO NMs), an increase in acetylcholine esterase (AChE) and Glutathione S-Transferase (GST) activity with increasing feeding rate was attributed mostly to zinc ions [14]. Due to inhibition of target sites such as GSTs, cytochrome P450-dependent monooxygenases, AChE, GABA-gated chloride channels, and octopamine, a neuromodulator, monoterpenes in essential oils also contribute to the incapacitating effects of essential oil-based NPs on insects [15]. After treatment with Bt-coated ZnO-NPs, the activity of cysteine protease, a-glucosidase, GST, and a-amylase in *Callosobruchus maculatus* was reduced [16].

iii. Impact on reproduction and development

Negatively charged NPs can disperse throughout the body and may stay in the ovaries of treated female larvae even during metamorphosis [17]. NPs in feed reduced female fertility because they disturbed oogenesis and induced ovarian anomalies, hence lowering egg-laying ability [18]. *Blattella germanica* nymphs subjected to aerosolized gold nanoparticles (AuNPs) reached adulthood more quickly, but ootheca viability was reduced, which the researchers hypothesised was due to stress caused by ingesting AuNPs. Stress may divert energy from the production of juvenile hormones, so diminishing vitellogenesis. Adults had a gold content of 12.7 9.4 lg/g, whereas nymphs had a gold content of 0.4 lg/g [19]. Titanium dioxide nanoparticles (TiO2-NPs) or zinc oxide nanoparticles (ZnO-NPs) decreased sperm bundles and testis weight in male *Agrius convolvuli* via phagocytosis [20]. By increasing ROS activity in the testis, they may also inhibit proliferation of germline stem cells [21].

iv. Impact on midgut

After ingestion, coated and uncoated NPs move through the midgut in unique ways. In *Drosophila* investigations, naked poly (D, L-lactic-co-glycolic acid) (PLGA) nanoparticles (NPs) encapsulated with BODIPY (boron-dipyrromethene, a fluorescent dye) went through the whole midgut without issue. On the other hand, Chitosan-coated PLGA NPs were shown to be retained in the posterior midgut for up to 10 hours. When they reached the acidic middle midgut, they created a substantial positive charge and were found to promote cellular uptake and mucoadhesion in the region [22]. As demonstrated in bumblebee workers exposed to nonlethal concentrations of silica NPs, which disrupted body temperature regulation, ingested NPs can have detrimental effects on the midgut and result in decreased food intake [23]. AgNPs increased oxidative stress in *D. melanogaster* midgut cells and elevated the synthesis of endoplasmic reticulum- and mitochondria-localized proteins hsp70 and hsp22. In addition, they activate caspase, which regulates programmed cell death, membrane instability, and the potential loss of the mitochondrial membrane [24].

v. Genotoxicity

In gene expression studies, unique molecular markers are utilised, allowing for accurate biomonitoring. After ingestion, AgNPs are linked to DNA damage in the salivary glands, gut, and brain, as indicated by gamma-H2AX (a biomarker for double-stranded DNA breaks), possibly due to ROS production and apoptosis [12]. AgNPs were shown to downregulate CrL15, a gene that regulates ribosome assembly, and upregulate CrGnRH1 and the Balbiani ring protein gene (CrBR2.2) in *Chironomus riparius*, indicating interference with protein synthesis and immunity [25]. Nonylphenol-NPs increased the mRNA expression of the ecdysone receptor, whereas AgNPs may either upregulate or downregulate it [26]. In contrast, [27] discovered that AgNP-treated Drosophila did not exhibit carcinogenic activity in the wing somatic mutation and recombination test, indicating that NPs may behave differently in vitro and in vivo.

Table 1: Showing tested nanoparticles of different metal ions and their effects on insects.

Tested nanomaterial	Insect target	Morphological damages and/or mode of action	References
Au nanoparticles (500–1000 µl)	*Aedes aegypti, beetles, and mealybugs*	➢ Triggered trypsin inhibition.	[28]
Carbon-dot-Ag nanohybrid (LC50 values from 0.30 to 0.76 ppm)	*Anopheles stephensi and Culex quinquefasciatus*	➢ Deformation of the larval body by affecting cuticle and cellular organization.	[9]
Graphene oxide nanoparticles (0.1 µl per 100 mg of insect's body weight)	*Acheta domesticus*	➢ catalase and glutathione peroxidases. ➢ heat shock protein (HSP 70)	[29]
Nanostructured Al_2O_3	*Sitophilus oryzae*	➢ Bind to the cuticle of the beetle as a result of turboelectric forces, absorbing its wax coat as a result of surface area phenomena, and causing insect dehydration.	[4]

Au: gold, Ag: silver and Al: aluminium.

Synthesis of Nanoparticles

The production of metal NPs is predicated on the development of an oxidation reaction in which metal ions (e.g., Ag+) are reduced by interaction with a reducing agent and transformed into neutral atoms (Ag0). This reaction can be mediated by many reducing agents as well as physical, chemical, and biological mechanisms [30].

Physical methods for the synthesis of silver nanoparticles, such as mechanical or ball milling, evaporation-condensation, and laser ablation [31], can be used; however, the required equipment requires a great deal of space, consumes a great deal of energy, and the yield of the final product is low, making it unsuitable for large-scale production.

Chemical methods for the synthesis of metal nanoparticles are the most prevalent; they depend on reducing chemicals (such as sodium citrate, potassium bitartrate, and sodium borohydride) to promote the reduction of metallic ions, the formation of nanoparticles, and the stabilisation of active ingredients (such as humic acid, polyvinylpyrrolidone, alginate, and chitosan) to prevent particle aggregation [32].

Biological synthesis, often known as biosynthesis or green synthesis, is the process of turning metallic ions into metal nanoparticles using plant extracts, microorganisms, or proteins. Many organic compounds, including as flavonoids, polyphenols, vitamins, proteins, terpenoids, and catechins, can act as reducing agents in the oxidation-reduction reaction of metal ions [33]. The biological manufacturing of nanoparticles is preferred over physical and chemical approaches because to its rapid syn-

thesis, higher control over size and shape, lower toxicity, cost-effectiveness, and eco-friendly approach. Another advantage is the stability of the generated particles. In biological synthesis, nanoparticle generation and stabilisation occur simultaneously because the reducing agent, in addition to synthesising the nanoparticles, can also contribute to their stabilisation, a process that needs the addition of particular compounds in other synthesis methods. This occurs because biomolecules in the reaction medium are adsorbed onto the surface of the nanoparticles, forming an outer layer known as the corona, which aids in particle stabilisation and/or enhances biological action and/or biocompatibility [34]. And among green synthesis processes, fungi are chosen over bacteria because fungi produce a superior biomass and do not necessitate additional extraction stages. Compared to plant extract, mycelial mass of fungi is more resistant to agitation and pressure, and appropriate for large-scale synthesis. Fungi are also extremely tolerant to metals, easy to handle, have a metabolism that produces mycotoxins, and create enormous numbers of proteins and enzymes.

A disadvantage of biological synthesis is the likelihood of fluctuations in the amounts of biomolecules present in the biological source (whether microorganisms or plants) due to variations in the environment or the availability of nutrients. To minimise interferences and increase nanoparticle production, rigorous scientific procedures must be utilised to evaluate the effect of synthesis reaction parameters such as pH, temperature, stirring speed, silver concentration, and amount of fungal biomass [35-37]. In spite of this, biologically generated silver nanoparticles have a wide range of applications. This field of research is being strengthened by the hunt for novel agents with reduced capacity and new applications, making it easier to comprehend and develop.

These bacteria can assist synthesis in either the intracellular or extracellular media, depending on the species engaged in the synthesis reaction. The nanoparticles are generated within the cells because the bacteria must come into direct contact with the silver ions that will be absorbed and digested within the cells during intracellular synthesis. Small, stable nanoparticles are produced by microorganisms capable of this synthesis; however, additional procedures are required to separate the nanoparticles from the cellular structures, which may be inconvenient [30].

Using either microbial biomass in direct contact with silver or microorganism extracts, extracellular production is possible. Due to the presence of carboxylic or amine groups on the cell wall surface, extracellular synthesis driven by microbial biomass occurs through the interaction of silver ions with proteins on the external surface of the microorganism's cell wall. Similar to intracellular synthesis, the nanoparticles adhere to the surfaces of the microorganisms, necessitating the need of separation techniques to separate the nanoparticles, which can increase material processing costs [38]. The activity of biomolecules contained in these extracts, which are derived from the microorganisms' biomass, results in the formation of metal nanoparticles from aqueous microbe extracts. As a result, nanoparticles form in an environment devoid of undesirable substances. This is the most frequent method for producing metal nanoparticles using microorganisms.

Depending on the microorganism or strain chosen, the method by which bacteria and fungi manufacture silver nanoparticles varies. Certain fungus and bacteria, such as *Fusarium oxysporum*, produce extracellular silver nanoparticles using NADH-dependent reductases; however, not all fungi use this enzyme. Additional biomolecules produced by these bacteria, such as coenzymes, naphthoquinones, and anthraquinones, can also aid in the reduction of silver. Hence, depending on their metabolism, distinct microorganisms can interact with a certain metal in a variety of ways. The complete method by which microorganisms produce silver nanoparticles is yet unknown [39].

Characterization of nanoparticles

To appreciate the internal and external structure of green synthesised NPs, the material's composition, size, structure, physical and electrical properties, size distribution, degree of aggregation, surface charge, and surface area must be characterised [40]. Seeing the colour change that has occurred in a flask containing fungal nanoparticles vs the control flask, which has no colour change, is the easiest way. Here, various types of metal ions produce distinct hues. Using a variety of characterisation methods, the surface chemistry and surface ligands of NPs are analysed. The ratio of surface area to volume has a substantial impact on the properties of nanoparticles. The X-ray diffraction method can be used to determine the nanoparticles' crystal structure and crystallite size. X-ray diffraction is based on the diffraction of x-rays by the periodic crystal lattice. Ultraviolet and visible (Uv-Vis) absorption spectroscopy is the alternate technique for examining nanoparticles. With this method, we may measure how much light is attenuated as it passes through a substance under study or after it has been reflected from a sample. UV and visible light are both energetic components of light that can raise the energy levels of electrons. It adheres largely to the Beer-Lambert law, which stipulates that the amount of light absorbed by a sample is directly proportional to its concentration and route length. Scanning electron microscopy (SEM) is a type of electron microscopy used to observe and characterise surfaces. Scanning electron microscopy is one of the most prominent techniques for evaluating and assessing the micro- and nanoparticle imaging properties of solid objects. The SEM's resolution of 10 nm or 100 is one of the reasons it is utilised for particle size analysis. Advanced models of these instruments have a resolution of approximately 2.5 nm (25) [41]. Transmission electron microscopy (TEM), a high-resolution measurement technique that produces magnified images by sending an electron beam through a sample of NPs, is the optimal method for analysing the size distribution, particle size, grain size, and morphology of NPs [42]. It produces high-quality, two-dimensional images that may be expanded or magnified and have a resolution of 1 nm. It is the appropriate instrument for characterising the chemical and structural content of the nanoscale. Crystalline NPs may be spherical, flat, cylindrical, tubular, conical, or irregular, with a uniform or uneven surface and a crystal-like surface. Using Fourier Transform Infrared Spectroscopy, the chemical surface of nanoparticles is easily detectable (FTIR). FTIR spectra were applied to determine the functional groups and various metabolites of the substance. These metabolites, which are positioned on the outside of NPs, may be responsible for NPs' stability [42]. X-ray diffraction analysis (XRD) is a technique used in materials science to determine the crystallographic structure of a substance. The material is subjected to incoming X-rays during XRD, and the intensity and scattering angles of the X-rays that depart the material are subsequently measured. As a result of its ability to interact with the electrons in an atom's inner shell, it has become the most popular and efficient technique for characterising nanoparticles. Using light scattering techniques such as dynamic light scattering, the size of nanoparticles can be assessed, as well as their stability over

time in suspension at varying pH and temperatures. Zeta potential is employed to evaluate the surface charge of nanoparticles and get insight into their stability and interactions with other molecules on their surfaces (Figure 2).

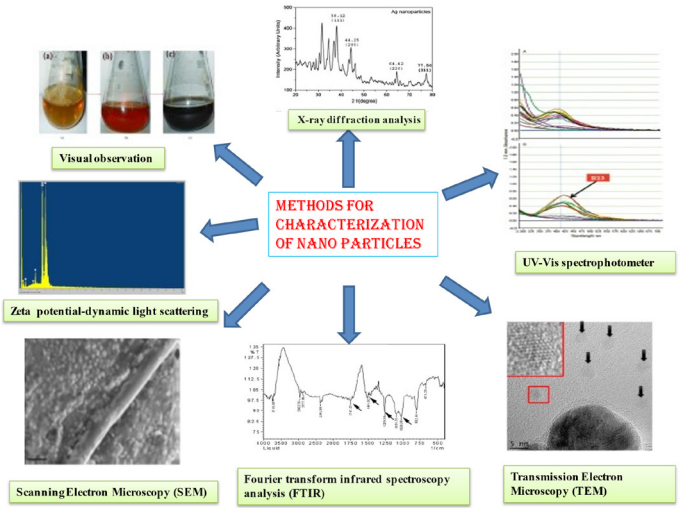

Figure 2: Showing different techniques used for characterizing nanoparticles.

Fungal nanoparticles in pest control

The insecticidal effect of silver nanoparticles synthesised with entomopathogenic fungi has been tested primarily against urban pests and human pathogen vectors, including *Aedes aegypti* mosquitoes (dengue, zika, and chikungunya vectors), *Anopheles stephensi* (malaria vector), and *Culex quinquefasciatus* (lymphatic filariasis vector) [43-45]. No studies on the application of AgNPs synthesised with entomopathogenic fungi for the control of agricultural pests have been found in the indexing bases of publications; however, studies have already demonstrated the potential of biogenic silver nanoparticles for the control of insects that cause damage to agricultural crops, such as *Spodoptera littoralis* (Lepidoptera: Noctuidae) [46], *Spodoptera litura* [47].

Thus, silver nanoparticles synthesised with entomopathogenic fungi may be a viable alternative for pest control in agricultural crops; however, additional research is necessary to fill the knowledge gap about this type of particle, such as determining whether there is a synergistic relationship between the fungi metabolites and silver in the action against insects and how it can be manipulated for agricultural use. In addition, a greater understanding of the toxicity of these chemicals to insects and non-target creatures, as well as their environmental impact, is necessary to promote their use in agriculture. In addition, in order to assure the safety of their application, it is necessary to create ways for getting more knowledge regarding the toxicity of these nanoparticles.

Toxicity assessment of nanoparticles

For the characterisation of silver nanoparticles, low-cost and efficient in vitro toxicity assessment methods were used. Before scaling up production or conducting more detailed research, these approaches can be used to improve the physical and chemical properties of nanoparticles due to their speed. For testing the toxicity of silver nanoparticles on cells and nucleic acids (cytotoxicity and genotoxicity), as well as on plants (phytotoxicity), in vitro analysis techniques can be used [48].

Observing the impact of nanoparticles on the development of morphological changes in cells (via phase-contrast microscopy) and physiological processes (production of reactive oxygen species-ROS-evaluation of cell proliferation, change in mitochondrial metabolism) is possible using a variety of techniques. The MTT assay is one of the most popular methods for assessing the cytotoxicity of eukaryotic cells in cell cultures. The method involves observing the reduction of 3-(4,5-dimethylthiazol-2)-2,5-diphenyltetrazolium bromide to formazan, which is used to measure mitochondrial metabolism in cells [48]. The MTT assay is commonly used to assess the cytotoxicity of silver nanoparticles [49].

The International Organization for Standardization (ISO) proposes employing genotoxicity testing to verify the safety of nanomaterial-based goods. According to ISO, in vitro and in vivo analytical methods can be used to assess genotoxicity, with a major focus on mammalian cells, with in vitro methods being the most extensively employed, especially the micronucleus test and the comet assay, which are simple to conduct and offer consistent results.

The comet assay can be used to examine the DNA integrity of both prokaryotic and eukaryotic cells after they have been exposed to a hazardous substance. Due to its uncomplicated method, it is frequently employed in toxicological investigations and is used to assess AgNPs in animal and plant cells [50].

The genotoxicity of AgNPs can also be determined by monitoring their direct effects on plant cells. Because it is a sensitive model for detecting substances that can cause chromosomal abnormalities, the onion (*Allium cepa*) is the most widely employed indicator organism for this type of study. The approach involves exposing the plant's roots to the examined agent at varied concentrations and for a specified period of time, and then analysing and quantifying the meristematic cells of the roots under an optical microscope to detect any abnormalities [51]. The Ames Test is another in vitro genotoxicity test that can be used to evaluate AgNPs. This method investigates the fraction of genotoxicity by evaluating mutation induction in Salmonella typhimurium bacteria that have been genetically modified [52].

Phytotoxicity studies can be conducted by observing the growth of the seed (germination, root growth, and stem) or the production of deformities in the leaves over the duration of treatment to determine the impact of chemicals on plant development. As model organisms, several plant species, including zucchini, tomato, castor, oats, lettuce, and radish, can be used to study the phytotoxicity of silver nanoparticles. Methods that use lettuce (*Lactuca sativa*) as a model in phytotoxicity testing are typically used for the examination of a wide range of compounds because they are simple, inexpensive, and yield accurate results; lettuce is sensitive to the presence of factors that cause environmental stress [53].

In insect pathology, several insect species are frequently utilised as alternative models for evaluating insecticidal action in order to establish preliminary findings regarding the insecticidal nature of a chemical or product before extrapolating the bioassays to more complex organisms. *Galleria mellonella* (Lepidoptera: Pyralidae), *Drosophila melanogaster* (Diptera: Drosophilidae), *Bombyx mori* (Lepidoptera: Bombycidae), and *Tenebrio molitor* (Coleoptera: Tenebrionidae) are all commonly used as analysis models for the insecticidal action of substances and pathogens; however, there are no reports in the literature *G. mellonella, D. melanogaster*, and *T. molitor* were among the

model insects used to examine the toxicity of silver nanoparticles; *T. molitor* is simple to cultivate and manipulate, making it perfect for this application [33]. When selecting insect species, consideration must be given to the possibility of employing them as toxicity models or the occurrence of evolutionary proximity with other commercially significant insect species.

Different metal ions used to synthesise pest control EPF

i. Gold (Au) nanoparticles

Aspergillus niger produced AuNPs that were more efficient against *C. quinquefasciatus* larvae than those of *A. stephensi* and *A. aegypti*. After 48 hours of exposure to the AuNPs produced by *A. niger*, all *C. quinquefasciatus* larval instars showed 100 percent mortality [53]. *A. stephensi* larvae were shown to be more vulnerable to larvicide produced with gold nanoparticles of *Chrysosporium tropicum* fungus mortality [53].

ii. Silver (Ag) nanoparticles

All stages of *C. quinquefasciatus* larvae were shown to be more vulnerable to the silver nanoparticles generated by the fungus *C. tropicum* mortality [54]. The biocontrol efficiency of Ag NPs generated by isolates *M. anisopliae* was superior to that of *B. bassiana*, Bio Magic, Bio Power, and Bio Catch *on Rhynchophorus ferrugineus* [55]. *A. aegypti* and A. stephensi larvicidal potentials of silver nanoparticles produced utilising the fungus *Cochliobolus lunatus* have been observed [56,57]. Emphasised the potential of the *B. bassiana* mycelial extract, which has activated the produced silver nanoparticles, as a biolarvicidal agent against the dengue vector *Aedes aegypti*. Biosynthesized silver nanoparticles from the entomopathogenic fungus *M. rileyi* were found to be effective in suppressing the larval stage of *S. litura* on cabbage [58].

iii. Other metal ions

Kumaravel et al [58] demonstrated that bimetallic zinc oxide and titanium dioxide nanoparticles generated utilising *M. anisopilae* can be utilised to defend crops *from S. furgiperda* [59], shown that *Trichoderma viride*-formulated titanium dioxide could significantly limit the growth of *H. armigera*. *Rhizopus oryzae*-mediated green production of magnesium oxide nanoparticles (MgO-NPs) showed larvicidal and adult repellence action against *Culex pipiens* at extremely low concentrations [60,61]. Demonstrated that the addition of zinc and aluminium nano-metal oxides to *M. anisopliae* increased its effectiveness against *C. quinquefasciatus* larvae. *B. brongniartii* Fe0NPs demonstrated promise in the management of *S. litura* by generating a drop in glutathione-S-transferase activities throughout the infection phase, although antioxidant enzyme activities reduced during later infection periods [62,63]. Demonstrated that silicon dioxide nanoparticles (SiO2) mixed with *M. robertsii* are effective against Colorado potato bugs (*Leptinotarsa decemlineata*) and cabbage beetles (Phyllotreta spp.). Copper nanoparticles (CuNPs) produced from an aqueous extract of *M. robertsii* were found to be efficient against *Aedes stephensi, Aedes aegypti*, *Culex quinquefasciatus*, and *Trichoptera molitor* [64]. Selenium nanoparticles (SeNPs) generated using *Trichoderma* fungus culture filtrate were discovered to be an excellent larvicidal and antifeedant agent, and can therefore be utilised to control *S. litura* larvae [65]. Zinc oxide nanoparticles (ZnONPs) produced by *A. niger* were shown to consume an LD50 level of 12.63 ppm to suppress 1st instar larvae of white grub *Holotrichia* spp [66].

Conclusions

Recent study reveals that using fungi to biogenically produce metal nanoparticles offers a lot of advantages and that these materials have a promising future in a variety of agricultural and medical applications. The nanoparticles' stability is given by cappings produced from fungus. Depending on the type of fungus utilised, this capping may potentially exhibit biological activity, functioning in tandem with the impact of the nanoparticle core. It is feasible to construct nanoparticles with diverse physicochemical properties by utilising multiple fungal species and synthesising them under a variety of conditions, including temperature, pH, biomass concentration, and metal precursor concentration, among others. However, a number of obstacles must be solved before the use of fungi for biogenic synthesis may be considered effective. They include the need to know which fungus to use, its growth parameters, the need for sterile conditions, and the time necessary for fungal growth and synthesis to be completed. Scaling up can also present obstacles, such as the need for greater research into the mechanisms underpinning the creation of capping layers and their constituent compounds. The research that has been published to date suggests that the use of fungi for the biogenic synthesis of metal nanoparticles can lead to a vast array of potential uses, notwithstanding the need for more investigation into certain problems. These nanoparticles have a substantial amount of application potential in the control of insect pests.

Abbreviations

CYP450: Cytochrome P450; NSA: Nanostructured Alumina; UV: Ultraviolet; Nps: Nanoparticles; Agnps: Silver; Nanoparticles; DNA: Deoxyribose Nucleic Acid; SOD: Superoxide Dismutase; Zno Nms: Zinc Oxide Nanomaterials; Ache: Acetylcholine Esterase; GST: Glutathione S-Transferases; Bt: *Bacillus Thuringenisis;* Aunps: Gold Nanoparticles; ROS: Reactive Oxygen Species; PLGA: Poly (D, L-Lactic-Co-Glycolic Acid); BODIPY: Boron-Dipyrromethene, A Fluorescent Dye; HSP: Heat Shock Protein; NADH: Nicotinamide Adenine Dinucleotide; Uv-Vis: Ultraviolet And Visible; SEM: Scanning Electron Microscopy; TEM: Transmission Electron Microscopy; FTIR: Fourier Transform Infrared Spectroscopy; XRD: X-Ray Diffraction; MTT: 3-(4,5-Dimethylthiazol-2-Yl)-2,5-Diphenyl-2H-Tetrazolium Bromide; ISO: International Organization For Standardization; Mgo-Nps: Magnesium Oxide Nanoparticles; Fe^0NPs: Iron Nanoparticles; Sio_2: Silicon Dioxide Nanoparticles; Cunps: Copper Nanoparticles; Senps: Selenium Nanoparticles; LD: Lethal Dose; EPF: Entomopathogenic Fungi.

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