Nanotechnology Controling Plant Pathogenic Fungi

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Nanoparticles are materials with at least one or more dimensions at the scale of 1–100 nm. This definition has taken several materials to be named nanoparticles into account. Nanoparticles that are natural likewise occur in numerous forms, for example, oceanic salt sprays and volcanic dust.

Keywords: biosafety ; disease control ; essential oils ; fungi ; nanocarrier ; nanoparticle ; nanopesticides ; nanotechnology

1. Introduction

Agriculture plays a vital role by providing nourishment and serving as a source of income for many countries. It is the major source of livelihood for people in rural areas; about 86% of the rural people depend on agricultural cultivation [1]. Approximately 15–18% of crops losses occur as a result of animal pests, while weeds and microbial diseases cause 34 and 16% losses, respectively. Fungal pathogens cause about 70–80% losses in yield [1]. Approximately, there are 1.5 million species that are classified under the kingdom ‘fungi’ and these fungal pathogens are mostly parasitic and saprophytic in nature, causing different diseases in agricultural crops. Fungal pathogens may cause serious decreases in the yield of different crops worldwide each year [2][3]. Presently, disease control depends on the utilization of agrochemicals, for example, fungicides. Regardless of numerous favorable advantages, such as fast action, reliability, and high availability, fungicides can cause negative impacts on the non-target living organisms because of their toxicity and their systemic mode of action by disrupting the metabolite levels in the biosynthetic pathway of aromatic amino acids within the soil microorganisms, the development of resistance, and resurgence in the population of pests and environment [4][5]. Moreover, it is assessed and estimated that about 80–90% of sprayed fungicides are lost to the environment after or during their applications [5][6]. Accordingly, there is an urgent need to procure high-performance fungicides which are cost-effective and cause less negative impacts to the environment.

Nanotechnology has prompted the advancement of novel ideas and agriproducts having tremendous potential to address the aforementioned issues. Nanotechnology has progressed in areas of pharmacology and medicine, yet has not developed nearly as much in agricultural uses [7]. The utilization of nanotechnology in the agricultural sector is presently being investigated in the delivery of plant chemical, water, and seed control, nanobarcoding, transfer of genes, controlled release of agrochemicals, and nanosensors [8]. Many researchers have designed nanoparticles pertaining to different qualities, such as pore size, surface properties, and shape, in such a way that they would be utilized as protectants or for exact delivery through encapsulation, and adsorption of an active ingredient [8]. There is a possibility for nanotechnology in agriculture to create and give another age of fungicides and different active ingredients for fungal disease control in plants, as presented in **Figure 1** .

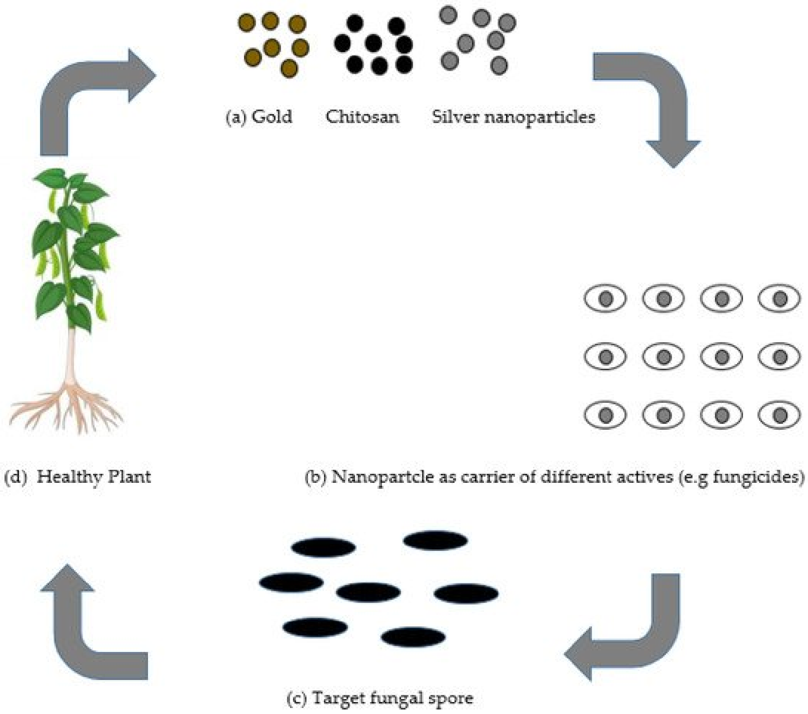

Figure 1. (**a**) Different nanomaterials as protectants used in plant protection. (**b**) Nanomaterials as transporters for several active ingredients such as fungicides. (**c**) Nanomaterials targeting different fungal pathogens. (**d**) The potentialities of nanomaterials to provide protection to the plant.

The application of nanoparticles for plants protection could be achieved using two types of mechanisms: (a) as individual nanoparticles giving protection in plants; or (b) nanoparticles as fungicides transporters of different active ingredients, for example, fertilizers, which could be applied by soaking/drenching or by spraying onto the foliar tissue, roots, or seeds. Nanoparticles serve as a carrier and may give numerous advantages such as (i) enhanced solubility of low water-soluble fungicides; (ii) enhanced shelf-life ; (iii) improved site-specific uptake in the targeted microbe; and (iv) reduced toxicity [9]. Other advantage of the nanocarrier system is the increment for the efficacy of the stability and activity of the nanofungicides under different ecological factors, essentially reducing the number as well as the quantity of applications, which accordingly diminishes harmfulness and decreases their expenses or cost.

Nanotechnology has played an important role in creating a footprint to develop several forms of formulations effectively. Up to now, nanotechnology in the area of agriculture has not reached its milestone because of the insufficient application of nanoproducts at commercial levels. Till today, only a few studies have been carried out in the fields. Therefore, there is a need for researches to shift towards the direction of testing on different crops, target fungus, and to carry out both short and long periods of field trials in order to make progress, and advances in the area of agronanotechnology. This work discusses the properties and synthesis of nanoparticles, new advancements in plant pathogenic fungal disease control by the use of nanoparticles alone as protectants, and nanoparticles as nanocarriers for fungicides. Moreover, using other nanoproducts such as agronanofungicides, Zataria multiflora , and ginger essential oils nanoformulations to control plant pathogenic fungi, as well as the biosafety and limitations of the nanoparticles applications, have been addressed. In this review, we have focused on the information that is more recent (last 5 years) and also on papers before 2016 (last 5tears), where we feel the information is not as rich. The majority of the papers discuss the synthesis, advantages, and efficacy of nanomaterials to control plant pathogenic fungi. Few papers discuss the phytotoxicity and limitations of nanomaterials applications.

2. An Overview on Nanoparticles

Nanoparticles are materials with at least one or more dimensions at the scale of 1–100 nm [10]. This definition has taken several materials to be named nanoparticles into account. Nanoparticles that are natural likewise occur in numerous forms, for example, oceanic salt sprays and volcanic dust [11]. Moreover, numerous viroid and viral particles fall in this definition of a nanoparticle. Natural nanoparticles are having different sizes and are irregular. Because of their huge surface-area-to-volume ratio and little size, they could be highly reactive and may absorb, bind, and convey mixtures of compounds, for example, DNA, small molecular drugs, proteins, probes, and RNA [12][13]. Besides the large surface area of nanoparticles, they also vary in different properties when compared with their counterparts. For example, gold is inert and is clearly a bigger structure, but could be reactive and reddish in color at the scale of nano size. Similarly, ZnO and TiO are usually found to be colorless at a nano size. Nanoparticles were found to melt at low temperatures and have high reactive potentials than their counterparts [13][14].

Nanoparticles are synthesized by various strategies and methods such as laser ablation, pyrolysis, emulsion, encapsulation, dispersion-precipitation, etc. [15][16]. New cycles and stages are developed quickly to the point that any portrayal is probably going to be obsolete soon. A lot of works have been carried out on the synthesis and preparation of nanoparticles in vivo by both microorganisms and plants [17]. The nanoparticles are categorized into several groups such as organic nanoparticles, inorganic nanoparticles, carbon base nanoparticles, and ceramic nanoparticles. The inorganic nanoparticles are further categorized into metal oxide and metal nanoparticles [18]. Likewise, carbon base nanoparticles are also further categorized into carbon nanotubes, fullerene, carbon nanofiber, carbon black nanoparticles, and graphene. These nanoparticles could also be grouped in terms of their dimension, such as two-dimension nanoparticles, three-dimension nanoparticles, and one-dimension nanoparticles [18][19]. The nanoparticles can be prepared by utilizing different approaches, e.g., bottom-up approach and top-down approach. The effects of different nanomaterials used and methods of their synthesis are listed in **Table 1** .

Table 1. Nanomaterials, methods of nanoparticles synthesis, effects and their uses.

Nanomaterial	Preparation Method	Advantages	Disadvantages	Effect	Source(s)
Organic					
Lipid Liposomes Lipopolyplexes Solid lipid nano-particles	**Chemical: sonochemisty, reverse phase evaporation High-pressure homogenization**	**It involves the use of less toxic compounds, and the delivery of DNA, xenobiotics, pesticides, essential oils, and transfection**	**It requires substantial energy for effective disintegration of high-solid waste**	**Cytotoxicity**	[20]
Carbon nanotubes, Nanofibers, Carbon nanospheres, activated carbon, nanodots, graphene oxide and graphene layer	**Arc-discharge, laser ablation, pyrolysis, chemical vapor deposition, and Carbonization**	**Biocatalysts, sensing, neural/orthopedic implants atomic force microscope probes**	**It requires the use of low pressure and noble gasses**	**Antimicrobial effect**	[21]
Synthetic Dendrimers (PAMAM, PPI) Polyethylene oxide Polyethylene glycol Polylactides Polyalklycyanoacrylates	**-**	**Delivery of therapeutic/ diagnostic agents, pesticides delivery of DNA/RNA**	**Short half-lives, and lack of targeting capability**	**Cytotoxic effect**	[22]
Polymeric Natural Cellulose, Starch Gelatin, Albumin Chitin, chitosan	**Chemical: suspension, emulsion, dispersion -precipitation**	**Biocompatible, biodegradable non-toxic for drug delivery delivery of DNA/RNA**	**Emulsions are thermodynamically unstable and therefore must be formulated to stabilize the emulsion from the separation of the two phases**	**Non-toxic/non-cytotoxic**	[23]
Inorganic					
Clay Montmorillonite layered double hydroxides	**Physical: exfoliation co-precipitation**	**Delivery of pesticides, fertilizers, plant growth promoting factors**	**-**	**inhibiting and synergistic effects**	[24]

Nanomaterial	Preparation Method	Advantages	Disadvantages	Effect	Source(s)
Metal nanoparticles AgO, TiO_2, ZnO, CeO_2; Fe_2O_3 FePd, Fe–Ni (magnetic); Silica; CdTe, CdSe (QDs)	**Physical: Arc-discharge, high energy ball milling, laser pyrolysis/ablation. Chemical: electrochemical, chemical vapor deposition sonochemistry, microemulsion sol-gel, reverse precipitation**	**Photothermal therapy, imaging studies, delivery of biomolecules (proteins, peptides nucleic acids), biosensors, diagnostic procedures, implants, pesticide degradation**	**It requires substantial energy for effective disintegration of high-solid waste, and the use of noble gas**	**Positive effect by promoting the growth of plants**	[20]
Magnetic type					
Magnetic nanoparticle	**Physical vapor deposition, mechanical attrition and chemical routes from solution**	**Photothermal therapy, Imaging studies, diagnostic procedures**	**special apparatus and formation of highly toxic gaseous as by-products**	**-**	[18]
Biosynthesized type					
Biosynthesized nanoparticles (Silver and gold nanoparticles, Ag & Au NPs)	**Ag^+ ion reduction by culture supernatant of *E. coli*, gold ions reduction by Bacterial cell supernatant (Pseudomonas aeruginosa)**	**Delivery of pesticides and fertilizers.**	**Generally lower biosynthesis efficiency and lengthier production time Downstream processing of intracellular products is more complex and expensive**	**Antimicrobial effect**	[25][26]
Nanocellulose and Cellulose nanocrystal	**-**	**Degrading of biomass/bio-waste from oil palm**	**It has limited flexibility, low thermal stability, brittleness and low crystallization rate, which hinders its use**	**No cytotoxic and ecotoxic effects**	[27]

PAMAM—polyamidoamine, PPI—polypropylene imine, QDs—semiconductor quantum dots.

Protectant nanoparticles are a material with a range of 10–100 nm; these nanoparticles have special structures and properties that are physically, biologically, and chemically unique [2][28]. Nanoparticles alone can be used on plant foliage, roots, or seeds for defense against different pathogens, such as fungi, insects, viruses, and bacteria. Nanoparticles that are metallic, such as copper, silver, titanium dioxide, and zinc oxide, have been widely investigated for their antifungal and antibacterial characteristics [29][30][31][32].

Chitosan is also a well-known nanoparticle with suitable biological characteristics, for example, biocompatibility, non-allergenicity, antimicrobial action, and biodegradability having low-toxic effects on humans and animals [33]. It also has the ability to actuate resistance to viruses in different tissues of plants by supporting them to resist several infections brought about by the mosaic virus of snuff, peanut, alfalfa, cucumber, and potato [34][35]. Nanoparticles of chitosan have possessed a significant antifungal characteristic, for example, controlling, tomato root rot, Botrytis bunch rot (grapes), P. grisea (rice plant), and Fusarium crown [36]; however, they are less effective against bacterial pathogens [29]. Antiviral activity has been observed on tobacco necrosis virus, tobacco mosaic virus, and bean mild mosaic virus [37]. Chitosan nanoparticles are promising as they appear to have a huge potential as nanocarriers [2].

3. Prospectives of Nanoformulations in Managing Plant Pathogenic Fungi

The production of essential oils in plants is mainly for defense purposes against pathogenic microorganisms [38]. Essential oils have many benefits such as quick decomposition and with broad antifungal spectrum compared to conventional fungicides, low toxicity, and bioaccumulation. Nanoencapsulation is a nanocarrier system that is used for the encapsulation of bioactive substances [39]. It can improve the antifungal efficacy of bioactive compounds (essential oils) by the increase in cell interactions among the microorganisms and nanoparticles, because of the small size which improves the cellular uptake. Nanoencapsulation in solid lipid nanoparticles (SLNs) is an efficient technique that enhances the application of essential oils as an antifungal agent [39][40]. SLNs are novel drug delivery systems for cosmetic and pharmaceutical drug active ingredients [41]. SLNs have unique properties, such as a large surface area, high drug loading, and small size. Their sizes are in the range of 50–1000 nm. SLNs can improve the solubility of essential oil(EO) in water, protect the EO against environmental conditions such as light, oxygen, acidity, and moisture, improve the controlled release of the EO, and increase the bioavailability of entrapped bioactive [41].

The availability of phenolic compounds such as Carvacrol and Thymol are the major constituents of Zataria multiflora essential oil that inhibit the growth of Aspergillus flavus fungus. This essential oil nanoemulsion has a very strong anti-fungal activity with minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of 100 ppm, respectively [39]. Based on these results, ZEO is an appropriate and potentially natural alternative for managing A. flavus [39]. In another study, the in vitro study had also shown a sustained and controlled release of Z. multiflora essential oils (ZEO) for 40 days. The strong activity of ZEO, after being encapsulated in chitosan nanoparticles (CSNPs) under both in vivo and in vitro conditions in comparison to the unmodified ZEO, was observed on the fungus B. cinerea [40]. The in vivo study had also revealed that the encapsulated Zataria essential oils at the concentration of 1500 ppm had shown a promising activity by decreasing both the disease incidence and disease severity of Botrytis -inoculated strawberries within the 7 days of storage at a temperature of 4 °C. This was then followed by two to three more days at a temperature of 20 °C. These findings have unveiled the important role of CSNPs that served as a controlled release system for Zataria EOs in order to enhance antifungal efficacies [40].

The delivery system of Eos, such as microemulsions, nanoemulsions, liposomes, and solid lipid nanoparticles, are designed for enclosing different compounds (natural bioactive) to improve antifungal efficacy [42][43]. Nanoemulsion is the dispersal of nanoparticles comprising of two different fluids that are insoluble, specifically water and oil, one of which is dispersed by a surfactant, as presented in **Figure 2** . Surfactant is needed in order to develop a formulation of nanoemulsions for interfacial layer rigidity, droplet quality under 100 nm, and droplet size reduction [44][45]. The utilization of EOs is very much designed to make explicit qualities implied for suitable uses [46][47] to manage the diseases of fungi. The decrease to the nanometric scale of the drop size could increase the zone of the substrate which then creates contact with the fungal pathogen to bring about cell death and lysis. The constituents of EOs can get to the pathways of the cell membrane due to their surface-to-volume proportions, physical characteristics, sizes, degrees of selectivity, and chemical stabilities, consequently setting the movement of EOs to arrive at their target areas [42].

Figure 2. (**a**) Combination of ginger essential oil (EO), water, and emulsifier (tween-80). (**b**) The final product of ginger EO nanoemulsion after the formulation. (**c**) The application of ginger essential oil (EO) formulation is promising and could be utilized to protect the plant from fungal infections.

The encapsulation innovations and controlled techniques for discharge have changed the utilization of nanotechnology-based ginger EOs as an antifungal and antibacterial for the conservation of different crops [48]. It is an effective delivery framework that may only be delivered when it is required, bringing about more prominent conservation of crops and lowering the costs of crop cultivation [49]. Some industries worldwide are aiming at formulating nanofungicides for conveyance by means of nanoencapsulation into the target tissue of plants. Numerous formulations are being developed to contain nanomaterials. The materials dissociate in water in order to improve their efficiencies [50]. Accordingly, they are utilized as nanoscale particles that comprise antifungal nanoparticle suspension that could be advantageously mixed with various media such as liquids, creams, and gels [50]. Previous investigations had uncovered that diverse nanoparticles could affect different pathogens. Thus, it is important to utilize nanoscale to develop new formulations from natural products, such as Eos, for fungicides [51]. Nanoemulsions that contain citral-EOs can disturb and enter the lipid structure of the cell wall (fungi). It brings about cell membrane annihilation and protein denaturation; this is followed by conformational changes, cell death, and cytoplasmic leakage. An effective system of delivery of useful particles from EOs would work in the treatment of fungal diseases of plants [51]. Mahdavi et al. [52] uncovered that polymeric nanofibers containing ginger EOs showed the consistent and nonstop delivery of the successful compound of EOs loaded onto the nanofibers which become a remarkable tool to control plant pathogens.

4. Biosafety of Nanoparticles

This is a principal concern in the utilization of nanotechnology in the management of fungal diseases in plants [53]. A few uncertainties exist with respect to the long-term impact of utilizing nanofungicide formulations on human health and the environment [54][55][56][57]. Consequently, there is a need to assess the chance of inhaling the nanofungicide at the time of spray by the farm laborers. Shi et al. [58] studied the toxicity of chlorfenapyr (nanopesticide) on mice and expressed that the chlorfenapyr formulation at 4.84–19.36 mg kg −1 showed less toxicity than the conventional formulation on the mice. Hence, nanoformulation could lessen the impact on the environment and humans than the conventional fungicide [59][58].

Nanoformulations are seen to be safer and friendlier to the environment in disease control, yet a high level of NPs toxicity incidentally delivered to the environment could cause negative effects on other microbes and man [59]. The toxicological impacts of nanomaterials on soil microbes and plants have been generally studied. Notwithstanding, the nanotoxicity impacts of plant–soil systems of interaction are still not generally known [59]. There are numerous knowledge gaps on the agroecotoxicity of NPs; more so, there are numerous uncertain issues and new difficulties concerning the biological impacts. Mousa et al. [58] stated that there is a need to study the phytotoxic effect of seeds that are exposed to various concentrations of NPs; this involves the phytotoxicity investigation on germination, root length, and NPs uptake within the plant systems [60][61]. The application of nanosized silica-silver particles in the field helped in managing powdery mildew disease in cucurbits; about 100% disease management was obtained at 21 days after the application [62]. The NPs were discovered to be phytotoxic at a high concentration (3200 ppm) when applied in pansy and cucumber plants. Comparative investigation to convey the NPs to the target location of an infected plant was conducted by Corredor et al. [63]. The impact of NPs on various species of plants differs, and both the negative and positive impacts of this, have been discovered. The NPs may cause negative and positive impacts [64][65][66] on the root extension, which depends upon the species of plants (cucumber, soybean, corn, carrot, tomato, and cabbage). TiO 2 and ZnO manufactured nanomaterials (MNMs) affected the microbe's community, biomass, and their diversity in the soil. Together, such reports infer that the soybean that is exposed to MNMs may be directly affected or via interaction of plant and microorganism, which includes nitrogen-fixing symbioses association that is sensitive to some metals [67][68]. Again, the phytotoxicity investigations on D-Zn-Al-LDH and H-Zn-Al-LDH were carried out on the seedlings of oil palm, and the results showed that both had the potential to reduce the phytotoxic impact when compared with their conventional counterparts [59]. To understand the potential advantages of applying NT to agriculture, the initial step to determine the transport and penetration of NPs in plants is required [69]. Since nanomaterials are brought into the soil because of human activities, they can penetrate the soil through the biosolids amended soils and atmospheric routes. The transport and penetration of NPs in the entire plant were assessed by Gonz_alez-Melendi et al. [70]. The findings indicate the potential of NPs to deliver different substances that are inhibitory to the plant fungal pathogens. Many works are required to explain the interaction between plants, phylloplane microflora, nanomaterials, soil micro-organisms, and endophytes, as well as both pathogenic and beneficial effects on the health of plants. Moreover, further investigations are required in order to develop bioindicators that would not only evaluate the effect of NPs on the environment, but also recommend different designs as well as models for the evaluation [54].

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