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Review Article

Phytosynthesized Nanoparticles as Novel Antifungal Agent for Sustainable Agriculture: A Mechanistic Approach, Current Advances, and Future Directions

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Due to rapidly changing environmental conditions, virulent pathogens have arisen continuously that invades the crops and badly affects their yield and quality of the cash crops which results in economic losses. To overcome the prevalent infection of fungal pathogens, there is an utmost need to develop alternative techniques that avoid conventional agriculture practices. The use of various chemical fungicides is not an environmentally sustainable solution to fungal diseases because it produces environmental contamination and is dangerous for human health. Nanotechnology provides solutions to disease control issues in a significant way. The scientific and industrial systems are being changed by this development. Similarly, nano-based instruments are highly promising in the agriculture sector, particularly for the production of powerful formulations that require appropriate distribution of agrochemicals, nutrients, pesticides/insecticides, and even growth regulators for improved efficiency of use. Nanotechnology provides an inexpensive, environmentally friendly, and alternative effective monitoring of agricultural fungal pathogens. Green nanotechnology is an innovative methodology that revolutionized the field of agriculture to solve these problems. Despite increasing plant growth, nanoparticles meet the agriculture demand for high yield. This study mainly focuses on the promise of various methods for the treatment of fungal diseases through nanoparticles.

1. Introduction

Agriculture contributes greatly in the production and revenue of agriculture for developing nations. It is the main source of income for rural people. Approximately, 86% of rural communities rely on agricultural production [1, 2]. Vegetable crops are important because these are the essential source of nutritious food and component of a balanced diet as they provide carbohydrates, fats, vitamins, and proteins [3]. Globally, around 18 percent crop losses are induced by animals in agriculture. The weeds and microbial diseases account for 34% and 16% losses, respectively. Fungal pathogens induced 70–80% of losses in crops due to the microbial pathogens [4]. Annual losses of crops through fungal pathogen are over EUR 200 billion [5]. An approximate 1.5 million species of fungi are found, and mostly these are parasitic and saprophytic fungal pathogens [6]. Fungal pathogens belong to the class Ascomycetes and Basidiomycetes which inhibit the growth of the plants that would result in substantial economic losses for farmers [7]. Chemical compounds that are used for degradation, spore suppression, and fungal pathogens are called fungicides. However, its use has risen due to its low prices and simplicity contributing to the overuse of chemical fungicides [8]. The overuse occurred in different strains of pathogens and impaired photosynthetic pigments, development, and reproductive organs of plants by changing metabolic and physiological activities. Moreover, they also deal with mitosis, cell respiration, and synthesis of microtubules. There is now a need to produce sources which are nontoxic, safe, and environmentally beneficial to prevent fungal diseases [9]. Synthetic chemical fungicides are poisonous and dangerous to human safety, climate, and soil biodiversity. The utilization of pesticides in Europe is around 45%, 25% in USA, and 25% in rest of the world. The use of such substances has detrimental impacts on the wellbeing of animals and soil microbes, contributing to adverse effects on soil fertility. Now, trends are progressing towards balanced, clean, and effective control of fungal diseases via nanoparticles [10]. In the 21st century, nanotechnology is recognized as one of the best innovation that intends to improve conventional farming practices and more environmental sustainability through better management and recycling strategies with reduced farm input waste [11, 12]. Nanotechnology includes the technologies of nanoscale that described the uses of atoms, molecules, or particles of submicron with chemical, biological, and physical systems which are 0.1–100 nanometers in size. Both organic and inorganic nanosized particles are used against viral, fungal, and bacterial pathogens. Plants take nanoparticles in the form of carbon, polymers, metal, and metal oxide nanoparticles and transfer through transport tissues to other plant organs. Nanoparticles are involved in improving the seed germination and plant growth and acts as antimicrobial agents [13]. Different biological materials are used for the formation of nanoparticles. These biological materials include microorganisms, reducing agents, plant extracts, and marine organizations [14]. Between these biological materials, plant extract is the most important biological material for the synthesis of nanoparticles [15]. Phytoextracts are used to synthesize the environmentally sustainable nanoparticles as they promote the plant growth, cause inhibition of fungal pathogens, and efficiently decrease crop diseases [16]. Researchers have shown that the green methods used for the synthesis of nanoparticles are more effective with less risks of loss, low costs, and ease of characterization [17].

2. Plant-Based Synthesis of Nanoparticles

The plant-based synthesis of nanoparticles is not a difficult procedure. In this approach, metal salts are mixed with plant extract and reaction is completed in minutes to couple of hours followed by color change of the plant base nanoparticles at room temperature. It undergoes further structural confirmation through various characterization techniques via a different spectrophotometry (Figure 1). Green synthesis is a suitable option that helps the environmental impacts to be reduced and helps to achieve the highest yield of nanoparticles implantation on a nanometric scale. Various studies have shown that green synthesis of metal nanoparticles contributed to the development of efficient and natural reducing agents [18]. In the synthesis of metallic NPs, the plant extract of Alfalfa sprouts was used for the first time [19]. The antimicrobial impact of molybdenum NPs has been associated to their small size and large surface to volume proportion, which enables them to combine perfectly with the microbial layers, and it is not solely due to the discharge of metal ions in solution [20]. Some investigations collected from the literature indicated that metal nanoparticles have been applied in plant agriculture as fungicides and germination stimulators [21]. Besides bacterial and viral pathogens, fungal plant pathogens are the main players that contribute to the severe loss of yield. Fungi cause enormous economic loss to agriculture, loss of food for consumption, and serious often fatal diseases in humans and animals. Molds and microscopic fungi have a great capacity to colonize on various kinds of substrates and to propagate under extreme environmental conditions. Disc diffusion method is a technique in which nanoparticles are used to evaluate the antifungal activity. In order to determine the antimicrobial activity of plant base nanoparticles, these can be analyzed by testing the microbe's inhibition region [22].

3. Mechanistic Insight of Antifungal Activity of Nanoparticles

The following events can be used to achieve the antifungal behavior in nonmaterial. Chitin, lipids, phospholipids, and polysaccharides with particular prevalence of mannoproteins and -1,3-D-glucan and -1,6-D-glucan proteins are generally included in the fungal cell wall and cell membrane composition [23]. Cell penetration is often the initial step in the stages involved in some microbial cell inhibition processes before other mechanisms are adopted. (1) Nanoparticles enter directly into the cell wall of fungal pathogen, (2) nanoparticles enter into the cell wall of fungal pathogen by ion transport proteins, and (3) unique receptor mediated absorption followed by internalization. First of all, nanoparticles enter into the fungal pathogen by three mechanisms which are under discussion [24] and cause membrane damage.

3.1. NPs Enter into Cell Directly (Membrane Damage). When fungal cells are exposed to nanoparticles (NPs), it leads to modifications in the structure of cell walls, such as surface shrinkage, clustering of cells, and formation of pits and pores. These changes, documented in various studies [25–31], are attributed to the direct interaction of NPs with fungal cell walls during the adsorption process. This interaction not only induces alterations in cell wall morphology but also affects inner membranes, causing distortions. Consequently, there are observable shifts in the

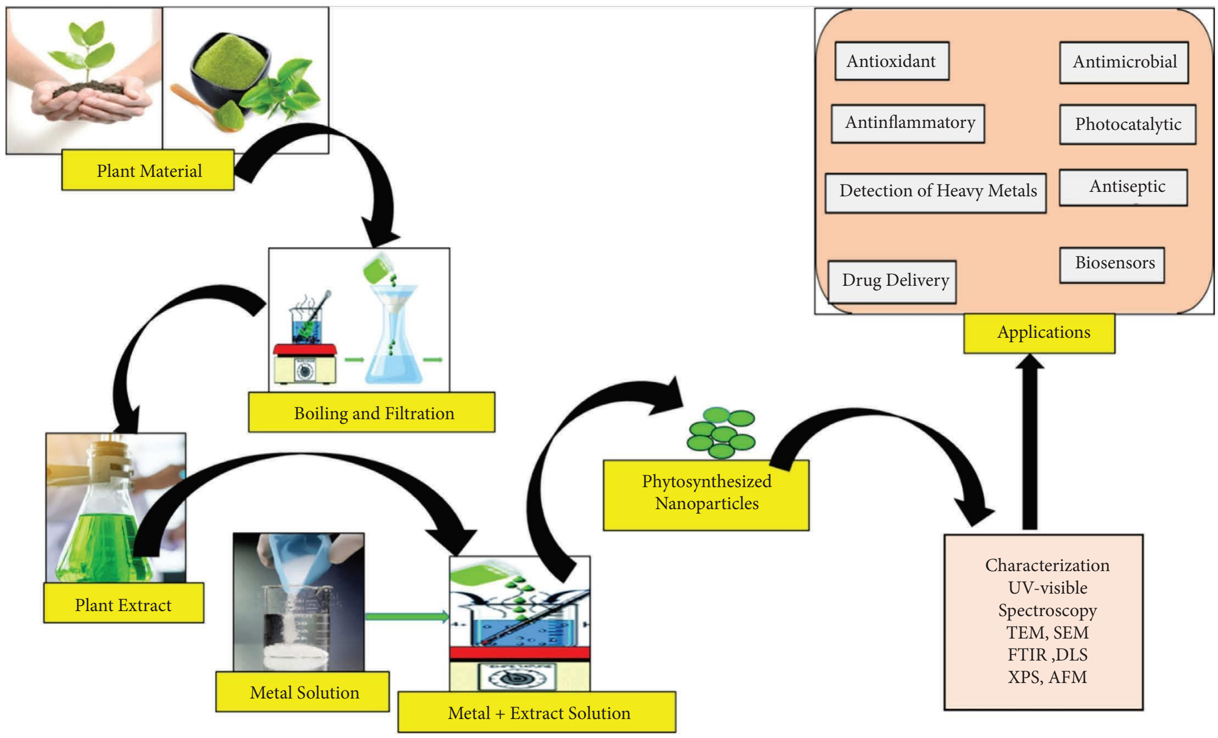

Figure 1: Plant-based synthesis of metal nanoparticles.

arrangement of organelles within the fungal cells, such as an increase in intracellular vesicles and vacuoles, alongside a reduction in cytoplasmic content [27, 32, 33]. Severe harm such as cell wall rupture or extensive damage does not necessarily occur upon exposure to nanoparticles. When nanoparticles come into contact with the outer membrane of a cell, they can engage with various components of the plasma membrane or the extracellular matrix. Subsequently, they can enter the cell primarily through a process known as endocytosis. This mechanism involves the incorporation of nanoparticles into small invaginations of the cell membrane, which then pinch off to create endocytic vesicles. These vesicles are then transported to specialized compartments within the cell responsible for sorting and trafficking. The specific type of endocytosis that occurs can vary depending on the type of cell and the particular proteins, lipids, and molecules involved in this intricate process [34, 35].

This discussion will delve into five distinct mechanisms of endocytosis: phagocytosis, clathrin-mediated endocytosis, caveolin-mediated endocytosis, clathrin/caveolae-independent endocytosis, and macropinocytosis. While some sources might classify the latter more four mechanisms as subtypes of broadly defined pinocytosis process, it is noteworthy that phagocytosis primarily transpires within specialized phagocytic cells, whereas the pinocytotic mechanisms are more prevalent and manifest in various cell types [36].

3.2. NPs Enter into Fungal Cell by Transporters. Nanoparticles phagocytosis is typically instigated through a process called opsonization. During opsonization, opsonins such as immunoglobulins like antibodies, complement proteins, or many other blood proteins (laminin and fibronectin) attach themselves to the surface of the NPs [37, 38]. Once opsonized, these NPs are identified and bound to phagocytes through specific interactions between ligands and receptors. This sets off a series of signaling events that can initiate the assembly of actin, the creation of extensions on the cell surface, and the subsequent engulfing and internalization of the particles. This internalization process results in the formation of what is referred to as a "phagosome" [39]. The timeline for these processes varies, taking anywhere from 30 minutes to several hours depending on factors like cell type and the characteristics of the particle surface.

3.3. Receptor-Mediated Absorption. The primary mechanisms for the penetration of nanoparticles (NPs) into the fungal cell are adsorption and diffusion. In the adsorption mechanism, NPs bind to the negatively charged groups of proteins, leading to protein disruption and cell death [40]. Additionally, there is evidence indicating the generation of reactive oxygen species (ROS) within pathogenic cells through the diffusion process [41]. Moreover, NPs can interact with various exposed groups on microbial surfaces, resulting in microbial destruction and inactivation, suggesting another potential mechanism [42, 43]. Phagocyte receptors, including Fc receptors, complement receptors, mannose/fructose receptors, and scavenger receptors, are implicated in facilitating these processes.

During our recent review, we explored various types of nanoparticles with distinct mechanisms of action that induce significant damage to fungal cells, particularly affecting fungal hyphae and spores. Notably, when fungi were exposed to AgNPs, ZnO NPs, or CuNPs, their hyphae underwent deformations, appearing both shrunken and distorted [29, 35, 44, 45]. Additionally, these nanoparticles led to alterations in growth patterns, resulting in clumping and thinning of hyphal fibers [26, 28, 32]. Interestingly, even in cases where CuNPs did not overtly impede fungal growth, hyphal structures displayed evident damage [35]. The consequences of hyphal damage were evident in the inhibition of mycelial growth by the nanoparticles, often in a dose-dependent manner [29, 32, 46, 47]. For instance, subinhibitory concentrations of AgNPs applied to *Candida albicans* led to the arrest of mycelial growth following the initiation of morphogenesis. Notably, the mycelia failed to elongate or form around the presence of AgNPs, in stark contrast to the healthy mycelial formation observed in the untreated control group [48]. In addition to hyphal deformation, the impact on spores and their germination significantly contributed to the antifungal effectiveness of nanoparticles.

Exposing spores to AgNPs during logarithmic mycelial growth stages revealed a reduced rate of mycelium growth upon germination. This phenomenon was concentration-dependent, where higher concentrations of AgNPs resulted in greater inhibition [49]. Similar effects were observed when ZnO NPs were applied to *Penicillium expansum*, causing damage to conidia and disrupting their developmental process, ultimately leading to the suppression of fungal growth [49]. Furthermore, ZnO NPs caused the formation of bulges on the surfaces of *Botrytis cinerea* hyphae, effectively impeding their growth [50]. An intriguing study highlighted that AuNPs interacted with fungal cell walls through electrostatic forces, subsequently releasing reactive oxygen species. This cascade of events interfered with intercellular signaling, induced cellular damage, and triggered apoptosis [51].

After internalization, nanoparticles can impair the enzyme-glucan synthase, thereby influencing the synthesis of N-acetylglucosamine [N-acetyl-D-glucose-2-amine] in the fungal cell wall. As a result of inhibition of the enzyme, anomalies such as increased cell wall thickening, cell membrane liquefaction, and the breakdown of cytoplasmic organelles, hyper-vacuolization, and cytoplasmic detachment from the cell wall can occur [32]. Nanoparticles interact with different biomolecules at molecular level and form complexes with various biomolecules, inducing structural deformation of biomolecules, catalytic protein inactivation, and nucleic acid defects such as DNA breakage and chromosomal aberrations [52]. The metal ion activates the ROS production and disrupts the biomolecules that contributes to the cell death [53]. As a result of ROS production, the lipid per oxidation expression is improved. Stress enzymes such as superoxide dismutase, glutathione dismutase, and ascorbate peroxidase have been upregulated/downregulated in the treatment of nanomaterials in fungi (Figure 2) [54].

4. Fungal Management by Green Nanotechnology

The inhibitory effect of various nanoparticles such as silver, copper, gold, and zinc nanoparticles rely heavily on their concentrations. Higher the concentration of nanoparticles, higher is the inhibition of fungal growth. Therefore, nanoparticles act as amazing antifungal agents that cause inhibition of fungal growth (Tables 1 and 2).

4.1. Zinc Oxide Nanoparticles (ZnO NPs). Zinc oxide nanoparticles (ZnO NPs) are extensively utilized nanomaterials due to their optical, photocatalytic, and antimicrobial characteristics [24]. When these nanoparticles are green-synthesized, their antimicrobial effectiveness becomes significantly greater than that of their chemically derived counterparts [94, 95]. Scientific literature indicates that phytosynthesized formulated ZnO NPs, acting as nanofungicides, exhibit notable antifungal properties against various fungal pathogens. The underlying mechanism of ZnO NPs in hindering microorganism growth involves inducing oxidative stress, which leads to the generation of reactive oxygen species, causing disruption to cell membranes through internalization, nanoparticle accumulation within cells, and impairment of nucleic acids [44]. Various studies are reported on phytofabrication of zinc oxide nanomaterials from different plant part extracts and used against various fungal pathogens. ZnO NPs prepared by leaves of strawberry are effective against Pathogenic fungi, *Botrytis cinerea.* ZnO NPs prevents the development of the pathogenic fungi *Botrytis cinerea* by changing the cellular function, which induced the fungal hyphae distortion [50]. ZnO NPs are prepared by the plant extract of *Parthenium hysterophorus*. These methods are environmentally friendly and have low cost. Different fungal pathogens have been checked for *Parthenium hysterophorus*. The ZnO NPs based on *Parthenium hysterophorous* induced a significant decrease in the growth of *Aspergillus niger* and *Aspergillus flavus* [96]. *Fusarium graminearum* is a fungal pathogen whose growth is prevented by use of ZnO NPs prepared from the bud extract of *Syzygium aromaticum* by strongly inhibiting the mycelia growth and mycotoxin development known as zearalenone and deoxynivalenol. This mechanism also increases the production of ROS, per oxidation of lipids, and decreased ergosterol value [97]. This alteration in macroconidia morphology such as rough, wrinkled, and shrinked surface expressed the antifungal activity. In order to determine the fungal pathogens of apple orchids, ZnO NPs were prepared by phytoextract of *Eucalyptus globules*. Highest zone of inhibition was observed at 100 ppm; for *Alternaria mali*, it was 76.3 percent; for *Botryosphaeria dothidea*, it was 65.4 percent, and for *Diplodia seriatait*, it was 55.2 percent. Manipulation of these NPs for fungal disease management could be used to preserve fruit crop [98]. One of the studies revealed the zones of inhibition for varied doses of

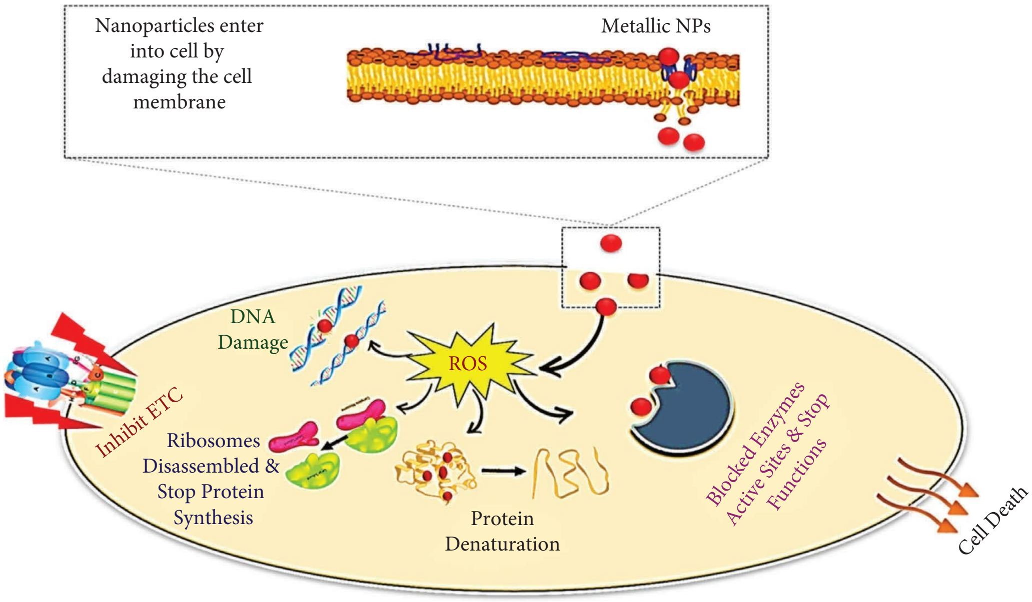

Figure 2: Mechanism of antifungal activity of nanoparticles.

ZnO NPs against *Candida albicans*. It is reinforced by the fact that as the concentrations of ZnO NPs rises, so does the zone of inhibition against fungal activity [56, 99].

4.2. Copper Nanoparticles. Copper nanoparticles (CuNPs) account for large number of implementations but few data are available. By using the leaf extract of *Moringa oleifera,* copper nanoparticles are fabricated [18]. Cu NPS are also formed through the common milk hedge-medicinal plant which is stem latex of *Euphorbia nivulia* [100]. Copper nanoparticles are also synthesized from the leaf extract of Aloe Vera (*Aloe barbadensis Miller*) [101]. Green synthesis of copper nanoparticles also formulates utilizing the peel extract of the pomegranate (*Punica granatum*) and Basil extract (*Ocimum sanctum*) in $CuSO_4$ solution [102]. Copper nanoparticles are formed through Citron juice (*Citrus medica*), and it is effective for inhibition of *Fusarium oxysporum*, *Fusarium graminearum*, and *Fusarium culmorum*. The more sensitive to copper nanoparticles was *F. culmorum* than *F. oxysporum* and *F. graminearum* [65, 103]. Copper nanoparticles can also be used in the field of plant pathology for the treatment of fungal diseases [104].

Copper nanoparticles have strong antifungal activity against a number of phytopathogens that includes *Curvularia lunata*, *Alternaria alternata*, *Fusarium oxysporum*, *Phoma destructiva*, *Phytophthora cinnamon*, *Alternaia alternata*, *Pseudomonas syringae*, *Penecillium digitatum*, and *Fusarium solani*. All of these phytopathogens are controlled by Cu NPs [105]. Copper nanoparticles prepared from the Clove (*Syzygium aromaticum*) bud extract shows strong antifungal activity against *Aspergillus flavus*, *Aspergillus niger*, *and Penicillium* spp. [66].

As compared to commercially available fungicides like bavistin, copper nanoparticles showed high inhibition of several fungal pathogens. There are different sources for green synthesis copper nanoparticles which are described by various authors by utilizing different plant extract. At varying doses, CuNPs demonstrate interesting antifungal properties (5, 10, 15, and 20 mM). At a dose of 20 mM (highest concentration), the maximum inhibition against *Alternaria* spp., *Aspergillus niger*, and *Pythium* spp. was 57.14, 63.81, and 58.05 percent, respectively, whereas the maximum inhibition against *Fusarium* spp. was 42.61 percent. The proposed mechanisms of action of metallic copper nanoparticles are based on changes in fungus cell structure and function; also, the nanoparticles damage structural DNA and disrupt its function, resulting in the death of the fungal microbe [106].

4.3. Silver Nanoparticles. In modern nanotechnology research, different reliable processes are developed for the synthesis of silver nanoparticles. One such process is green synthesis [107, 108]. Silver nanoparticles possess antimicrobial properties and they act as alternative for the development of natural antimicrobes [18, 108]. Different authors identified the processes for the green synthesis of silver nanoparticles via utilizing different plant extract. Silver nanoparticles are formed by using *Ananas comosus* (pineapple) juice [109]. The silver nanoparticles were also phytosynthesized extracellularly by using the leaf extract of *Ziziphora tenuior* [110]. The biosynthesis of silver nanoparticles by means of the Moringa leaf extracts has significant antimicrobial activity [111]. Silver nanoparticles are prepared by using the green chemistry technique against different fungal pathogens. Silver nanoparticles are prepared by using the leaf extract of *Acalypha indica*, and their potency is tested with different concentrations in order to determine the inhibitory effect against plant pathogens like *Alternaria alternata*, *Curvularia lunata*, *Macrophomina phaseolina*, *Botrytis cinerea*, *Sclerotinia sclerotiorum*, and *Rhizoctonia solani*, respectively.

Table 1: In vitro: antifungal potential and their mode of action of various phytosynthesized nanoparticles.

Plant name	Extract	NPs	Size and shape	Fungal strain	Inhibition zone/MIC	Mode of action	References
Beta vulgaris *Cinnamomum tamala* *Cinnamomum verum* *Brassica oleracea*	Leaves	ZnO	20 ± 2 nm spherical	*Aspergillus niger* *Candida albicans*	8 mm 8 mm	Inactivate the cellular metabolic activities by disrupting the cellular organelles. Disrupt the genetic material eventually causing cell death.	[55, 56]
Nyctanthes arbortristis	Flower	ZnO	20 ± 2 nm spherical	*Alternaria alternate* *Aspergillus niger* *Botrytis cinerea* *Fusarium oxysporum* *Penicillium expansum*	64 μg/mL 16 μg/mL 128 μg/mL 64 μg/mL 128 μg/mL	Interact with the outer surface of the plasma membrane when they come into contact with fungal cells. The structure of the plasma membrane is disrupted, and the permeability of the membrane is altered. The disruption of membrane structure and subsequent accumulation of NPs in the cytoplasm impede critical cell growth processes.	[57]
Salvia officinalis	Leaves	ZnO	26.14 nm spherical	*C. albicans* SC5314 *C. albicans* 4175 *C. albicans* 5112	13 ± 3 mm 14 ± 2 mm 11 ± 2 mm	The suppression of ergosterol production and the loss of membrane integrity appeared to be the origins of antifungal activity.	[58]
Cinnamomum camphora	Leaves	ZnO	21.13 nm spherical	*Alternaria* alternate	20 mg/L	Protein and nucleic acid leakage.	[59]
Pterocarpus santalinus	Wood	ZnO	15–25 nm spherical	*C. albicans*	9 mm 14 mm 20 mm 12 mm	Inactivate sulfhydryl groups which leads to produce insoluble compounds in cell wall and eventually degrade membrane-bounded enzymes, proteins, and lipids that cause cell death.	[60]
Ziziphus nu mmularia	Leaf	ZnO	17.33 nm Spherical Irregular	*Candida* spp.	9 mm 10 mm 11 mm 12 mm 14 mm 16 mm	Produce intracellular production-free radicals such as hydroxyl, singlet oxygen, superoxide, and nitric oxide that may enter into nuclear membrane and damage DNA which cause irreversible chromosomal damage eventually cell death.	[61]
Momordica charantia	Fruit	CuO	245 nm spherical	*Trichophyton rubrum*	31.66 mm	They have a stronger affinity for amines and carboxyl groups on fungal cell surfaces, and their huge surface area allows for better interaction with the fungus.	[62]
Bougainvillea glabra	Flower	CuO	5–20 nm spherical	*Aspergillus niger*	4-5 mm	Through their surfaces, penetrate into fungus' cell membrane and interrupt the cellular activities.	[63]
Celastrus paniculatus	Leaves	CuO	2–10 nm spherical	*F. oxysporum*	76.29 ± 1.52	Affect macromolecule DNA, its replication, and protein synthesis, leading to fungal death.	[64]
Citrus medica	Fruit	CuO	33 nm spherical	*F. culmorum* *F. oxysporum* *F. graminearum*	33 mm 28 mm 20 mm	Produce pits in the membrane, which cause cellular components to leak out and finally cause cell death. Oxidative stress appears to be on the rise.	[65]
Syzygium aromaticum	Bud	CuO	20 nm spherical	*Penicillium* spp.	6 mm	Enter the cell wall, causing cellular component leakage and, eventually, cell death.	[66]

Table 1: Continued.

Plant name	Extract	NPs	Size and shape	Fungal strain	Inhibition zone/MIC	Mode of action	References
Persea americana	Seed	Cu	42–90 nm spherical	*A. niger* *A. fumigatus* *F. oxysporum*	9 mm 11 mm 8 mm	Enzymes degradation and denaturation leads to cell death.	[67]
Falcariavulgaris	Leaves	Cu	20–25 nm spherical	*C. albicans* *C. glabrata* *C. guilliermondii* *C. krusei*	30.6 mm 30.8 mm 33.4 mm 34.8 mm	Inhibits fungal growth by producing ROS and causes hyphae lysis.	[68]
Cassia fistula	Leaves	CuO	2–38 nm spherical	*Fusarium oxysporum*	$91.9 \pm 0.16\%$	Deformation of fungal cell, membrane disruption, lipid peroxidation, protein, and enzymes denaturation.	[69]
Ligustrum lucidum	Leaf	Ag	13 nm spherical	*Setosphaeria turcica*	200 μg/mL	Fungal hyphae distortion was found.	[70]
Psidium guajava	Leaves	Ag	20–35 nm spherical	*Rhizopus oryzae* *Aspergillus niger* *Saccharomyces cerevisiae*	12.42 ± 0.11 mm 10.78 ± 0.18 mm 9.71 ± 0.21 mm	The cell became dysfunctional. NPs reach the cytoplasm and interact with sulfur-containing proteins and enzymes, interfering with DNA replication depending on the level of membrane damage. More easily access the cytoplasm or interact and disrupt cell membranes due to structural variations.	[71]
Panax ginseng	Roots	Ag	50–90 nm spherical	*F. graminearum* *F. avenaceum* *F. poae* *F. sporotrichioides*	47–51 μg/mL	Invading the fungal cell and causing damage to the cell wall and other cellular components.	[72]
Melia azedarach	Leaves	Ag	18–30 nm spherical	*Verticillium dahlia*	51 μg/mL	By destroying membrane integrity, affecting the function of membrane-bound enzymes involved in the respiratory chain.	[73]
Citrus limetta	Peel	Ag	18 nm spherical	*Candida albicans*	15 ± 0.75 mm	Cell blebs and a thick exudate deposition around the cell were induced by AgNPs, indicating intracellular material leaking.	[74]
Ocimum sanctum	Leaves	Ag	0–50 nm spherical	*Candida tropicalis* *C. krusei* *C. kefyr* *A. niger* *A. flavus* *A. fumigatus*	2 mm 1 mm 5 mm 3 mm 1 mm 2 mm	Interaction with cytoplasm resulted in cell membrane damage.	[75]
Allium saralicum	Leaves	Ag	20–40 nm spherical	*C. albicans* *C. glabrata* *C. parapsilosis* *C. krusei* *C. guilliermondii*	33.8 ± 0.44 mm 36.2 ± 1.3 mm 35.2 ± 1.3 mm 40.6 ± 1.34 mm 43.6 ± 1.14 mm	It has a number of compounds that work synergistically to prevent microbial infections. As a result, this causes significant harm to the fungal cell, resulting in its death.	[76]

Table 1: Continued.

Plant name	Extract	NPs	Size and shape	Fungal strain	Inhibition zone/MIC	Mode of action	References
Aloe barbadensis	Leaves	Ag	70 nm cubical, rectangular and spherical	*Aspergillus* spp. *Rhizopus* spp.	21.8 ng/mL	Silver nanoparticles harmed not only fungal hyphae but also conidial germination, induced various deformations such as cell membrane structure, and inhibited the normal budding process of both fungal strain most likely owing to the degradation of membrane integrity.	[77]
Malva parviflora	Leaves	Ag	50.6 nm spherical	*Helminthosporium rostratum* *Fusarium solani* *Fusarium oxysporum* *Alternaria alternate*	88.6% 81.1% 80.7% 83.0%	The nanoparticles were able to enter the plasma membrane, and hindered the normal functioning of proteins in the cell membrane, causing the cells to collapse.	[78]
Rhamnus virgate	Leaves Aqueous Ethanol	AgO AgO	Spherical, ~20 Cuboid ~22 nm	*Aspergillus flavus* *Aspergillus niger* *Mucor racemosus*	14.05 56.25 112.5	Interaction of fungal hyphae, mycelia, and spores leads to inhibition of fungal cell growth.	[79]
Croton sparsiflorus	Leaves	Ag	16 nm spherical	*Mucor* spp. *Tricoderma* spp. *Aspergillus niger*	0.1 cm 0.1 cm 0.1 cm	By interacting with electron phosphorous and sulfur-containing molecules like DNA, they penetrate within the fungus and cause harm.	[80]
Vetiveria zizanioides, *Cannabis sativa*	Roots, leaves	Au	10–35 nm spherical	*Penicillium* spp. *Aspergillus* spp. *Aspergillus flavus* *Aspergillus fumigates* *Fusarium* spp. *Mucor* spp.	34 mm 29 mm 34 mm 34 mm 29 mm 29 mm	May have diffused readily across the cell membrane to the interior of the cell, causing DNA synthesis, repair, and replication to be slowed, resulting in cell death.	[81]
Brassica oleracea	Flower buds	Au	12–22 nm colloidal	*Aspergillus flavus* *Aspergillus niger* *Candida albicans*	5, 7, 9 mm 5, 8, 9 mm 5, 7, 12 mm	It simply binds to the cell wall and causes damage and cell death.	[82]
Allium sativum	Cloves	Au	7–21 nm spherical	*C. albicans* *C. tropicalis* *C. crusei* *C. guilliermondii*	13.52 μg/mL 39.00 μg/mL 19.00 μg/mL 19.00 μg/mL	ROS generation altered fungal cell shape and morphology that leads to cell membrane damage and eventually cause cell death.	[83]
A. muricata	Leaves	Au	25.5 nm spherical	*A. flaws* *C. albicans* *F. oxysperium* *P. camemeri*	31 mm 42 mm 50 mm 66 mm	Direct contact with pathogens and cause DNA breakage and eventually cell death.	[84]

Table 2: In vivo plant model: antifungal potential and their mode of action of various phytosynthesized nanoparticles.

Plant name	Extract	Size and shape	NPs	Fungal strain	Mode of action	Plant disease	References
Trachyspermum ammi	Seed	15–20 nm cubic	ZnO	*Cercospora canesens*	Adsorb with pathogen surface, penetrate and damage the cell membrane and internal organelles	*Mung bean* (leaf spot)	[85]
Eclipta alba	Leaves	32 nm hexagonal	ZnO	*Sclerospora graminicola*	Directly NPs inhibit spore germination and indirectly enhance defense system which scavenge free radicals	*Pearl millet* (downy mildew)	[86]
Terminalia bellerica	Leaves	22 nm hexagonal	ZnO	*Alternaria brassicae*	Damage cell wall, induce stress in cell, disintegrate macromolecules, and degrade cytoplasmic material	Mustard crop (blight disease)	[87]
Citrus sinensis	Peel	32–47 nm spherical	AgO	*Modiolula phaseolina*	Restrict respiratory sequence which leads to cell death	*Faba bean* (charcoal rot)	[88]
Moringa oleifera	Leaves	450 nm crystalline	Ag	*A. flavus*	Inhibit DNA replication, inactivate of enzymes, degrade cellular proteins which leads ultimately to loss of enzyme expressions, and change the aflatoxins biosynthetic pathways	*Rice aflatoxins*	[89]
Azadirachta indica	Leaves	22–30 nm spherical	Ag	*Alternaria solani*	Disturb DNA replication, enzymes proteins denaturation, and inactivate normal cells functions	Tomato (early blight)	[90]
Cassia fistula	Leaves	2–38 nm spherical	CuO	*Fusarium oxysporum*	Membrane leakage, deformation of macromolecules eventually cell death	Tomato wilting	[69]
Eucalyptus globulus Mentha piperita	Leaves	65 nm triangular 45 nm cluster	Cu	*Colletotrichum capsici*	Arrest and inhibit mycelial growth	Chilli (fruit rot)	[91]
Tamarix aphylla	Leaves	50 nm spherical	CuO	*Fusarium oxysporum*	Inhibit growth by damaging fungal cells and induce plant responses against disease	Musk melon (fusarium wilt)	[92]
Curcuma longa	Rhizome	20–30 nm spherical	CuO	*Fusarium oxysporum*	Damage and deform the fungal cells membranes	Chickpea wiltting	[93]

Silver nanoparticles with a 15 mg concentration demonstrated excellent inhibitory efficacy against all these fungal plant pathogens [112]. $AgNO_3$ (1 mM) is mixed with different plant extracts like *Thevetia peruviana* seeds extract (10%) to synthesize silver nanoparticles and check their antifungal activity against different pathogens. In maize plants, leaf spot disease is common which is caused by *Curvularia lunata*. Exposing the inoculated samples to silver nanoparticles for 24 hours under sunlight and subsequent autoclaving methods resulted in approximately 95% inhibition [113].

Silver nanoparticles synthesized from the bark extracts of *Shorea tumbuggaia* and *Boswellia ovalifoliolata* and leaf extract of *Svensonia hyderobadensis* have strong antimicrobial activity against *Fusarium oxysporum*, *Rhizopus arrhizus*, *Aspergillus flavus*, *Curvularia lunata*, and *Aspergillus niger* by measuring their zone their zone of inhibition. In case of *Boswellia ovalifoliolata* and *Shorea tumbuggaia*, the silver nanoparticles are synthesized from their bark extracts and shows higher toxicity against *Aspergillus*, *Fusarium* and *Pseudomonas* species. However, the synthesis of silver nanoparticles from the leaf extract of *Svensonia hyderobadensis* exhibits strong antifungal activity against *Rhizopus* and *Pseudomonas* species [114]. Silver nanoparticles prepared from the leaf extracts of *Argemone maxicana* using $AgNO_3$ (5 mM) solution shows higher toxicity towards *Aspergillus flavus* [115]. Silver nanoparticles prepared from the leaf extract of *Svensonia hyderabadensis* by using $AgNO_3$ (1 mM) solution are effective against *Rhizopus arrhizu*, *Aspergillus Niger*, *Fusarium oxysporum*, and *Curvularia lunata*. Best antifungal activity was detected towards *Aspergillus niger* (11 mm), *Rhizopus arrhizus* (10 mm), and *Curvularia lunata* (10 mm), and less antifungal activity was noticed towards *Fusarium oxysporum* (8 mm) [116]. Silver nanoparticles are also prepared by means of different chemicals like $AgNO_3$ (1 mM) with *Thevetia peruviana* seeds extract (10%). Bright sunlight or autoclave method and sometime the combination of both are used. Color changes in NPs from the light milky white into dark orange, dark green, and dark brown are noticed during the synthesis of different nanoparticles. Relatively rare experiments are performed on silver nanoparticles in order to monitor the diseases of plants. The analyses of silver nanoparticles reveal that they have great impact on colonial development of spores and on the improvement against various diseases caused by plant pathogenic fungi. The interpretation of silver nanoparticles is higher in plants with preventive measures that can facilitate the direct interaction of the silver ions with the pathogen's spores and germ tubes and inhibits their survival. As a result, this strongly indicates that Ag NPs could have numerous substantial applications to control different diseases induced by plant pathogenic fungi [112]. *A. brasiliensis*, *C. globosum*, *P. pinophilum*, *P. variotii*, and *T. virens* were all inhibited by the AgNPs tested. As a result, AgNPs can be utilized to inhibit mold growth on building materials. The mold species' susceptibility to AgNPs varies. At a relatively low concentration of AgNPs (4.28 mg/l), total suppression of *P. variotii* growth was found [76].

4.4. Gold Nanoparticles. GNPs fabricated biologically by using multiple extracts of fresh leaves from *Diopyros kaki* [117], *Azardirachta indica* [118], *Mentha piperita, Pelargonium graveolens* [119, 120], *Moringa oleifera* [121], and *Artemisia dracunculus* [122] are reported. Nontoxic reducing agents are used in order to improve the affinity of gold nanoparticles. Various reducing agents were identified, with sodium citrate and sodium borohydride being the most widely used [123]. Many reducing and stabilizing agents are obtained from the plants [124]. Gold nanoparticles are important to inhibit numerous fungal pathogens. They are opening up a new door in the agriculture sector because of their antifungal properties. Different biosynthesized gold nanoparticles have an antifungal effect through various plant extracts that are described above, and it could be used against different plant pathogenic fungi. Gold nanoparticles are synthesized from *Abelmoschus esculentus* by using seed extract, and it shows antifungal activity towards different plant pathogenic fungi such *as Aspergillus niger*, *Aspergillus flavus*, *Puccinia graminis*, and *Candida albicans*. The highest inhibition zone was recorded for *Candida albicans* and *Puccinia graminis*. The growth of *Sclerotium rolfsii*, a soil-borne filamentous fungus, can also be prevented by plant-mediated gold nanoparticles using plant extract *Mentha piperita*. While gold nanoparticles synthesized by *Mentha piperita* were reported to exhibit high activity against A. flavus, their efficacy was found to be comparatively lower against Candida albicans. *Agaricus bisporus* is an edible mushroom; its extract with a concentration of 1–9 g DP/100 ml distilled water and 8–10 ml of $HAuCL_4$ solution is used to determine the antifungal properties of gold nanoparticles towards *Aspergillus flavus* and *Aspergillus terreus*. These green-synthesized gold nanoparticles show maximum inhibitory effect toward *Aspergillus flavus* as compared to *Aspergillos terreus*. The green synthesis of GNPs results in aqueous gold ions exposed to the leaf extract of *Salix alba* that display strong antifungal activity with *Aspergillus flavus* and *Alternaria solani* fungal strains. Even, at elevated temperatures, these GNPs are relatively unstable. Furthermore, excellent inhibitory effect of gold nanoparticles was shown against *Aspergillus solani* and *Aspergillus niger* and lowest inhibitory effect toward *Aspergillus flavus* [18]. The findings provide substantial evidence that Au NPs might be used as an antifungal drug that avoids the negative side effects and passive immunological responses of conventional biocidal treatments. As a result, it has been established that Au NPs have a high antifungal effectiveness and so have a great promise in medication formulations for antifungal ailments [125].

5. Conclusion and Future Directions

Fungal diseases are undervalued worldwide, although the fact is they pose a serious threat to plants, animals, and human health. According to increase in population pressure, there is a need to find ways to enhance the quality and yield of agricultural cash commodities. Although there has been an increase in the application of fungicides in agriculture, the introduction of pathogenic fungi that are fungicide-resistant has reduced the availability of many antifungal drugs. As a result, of this, innovative antifungal agents must now be developed. Recent significant advancements in this area have made using nanotechnology to discover novel nanofungicides a potential tactic. Green nanotechnology is considered a superior technology for management compared to other conventional methods. This approach significantly enhances food safety and improves quality and food processing by control of pathogenic fungi. It also minimizes the food nutritional losses and best alternative of chemical-based fungicides. Significant literature review identified possible attributes of plants extract-based NPs can improve the agriculture to overcome the adverse effects of toxicity. The present review has critically and thoroughly studied the antifungal efficacy of nanoparticles in agriculture. Remarkable progress has been made in the application of nanoparticles to control the fungus. As the studies have demonstrated, these nanoparticles might serve as an excellent substitute for chemical-based fungicides in agriculture. Due to their small size, NPs easily enter into pathogenic cells, interfering with their cellular materials like DNA and protein and leading to programmed cell death. Most of the monometallic nanoparticles are evaluated as antifungal agents. Therefore, it is necessary to analyze the bimetallic or trimetallic nanoparticles as well because they have significantly different properties from monometallic NPs. Based on the review, the majority of the investigations were examined in vitro. However, it is crucial to use the in vivo approach as future perspective to understand how fungi behave within the field study and there is no study reported on gold NPs application in field, as this opens up numerous possibilities for the use of nanoparticles in agriculture. However, other limitations, such as selectivity index, efficacy, and toxicity, require further investigation. To assess the effectiveness and safety, in vivo evaluation is also required. Despite the fact that some nanoparticles are restricted because they become harmful in high concentrations, if properly harnessed, they could soon find use in biological sciences. As a result, it can be said that this succinct review strengthens the already available knowledge about NPs and causes further research to confirm the future uses of NPs in the treatment of fungal diseases.

Abbreviations

NPs: Nanoparticles
ROS: Reactive oxygen species
DNA: Deoxyribonucleic acid
ZnO NPs: Zinc oxide nanoparticles
Cu NPs: Copper nanoparticles
$CuSO_4$: Copper sulfate
AgNPs: Silver nanoparticles
$AgNO_3$: Silver nitrate
Au NPs: Gold nanoparticles
SOD: Superoxide dismutase
POD: Ascorbate peroxidase
CAT: Catalase
TPC: Total phenolic content
TFC: Total flavonoid content
SEM: Scanning electron microscopy
TEM: Transmission electron microscopy
UV: Ultra violet
AFM: Atomic force microscopy
FTIR: Fourier transform infrared
DLS: Dynamic light scattering
XPS: X-ray photoelectron spectroscopy.

Data Availability

All the data are included in the manuscript.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Authors' Contributions

Javeria Parveen, Zia-ur-Rehman Mashwani, and Khafsa Malik devised the study. Javeria Parveen, Tahira Sultana, and Amir Ali wrote the first draft. Naveed Iqbal raja, Zia-ur-Rehman Mashwani, and Khafsa Malik provided guidance and supervision. Amir Ali and Abeer Kazmi, Abd Ullah, Bushra Qayyum, and Saif Ur Rehman edited and reviewed the manuscript. All the authors reviewed and endorsed the final version of manuscript for submission and publication.

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