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A Comprehensive Review of Metal-Based Fungicides and Effects on Agriculture

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Abstract

Plants are the main source of energy and organic matter through which soil ecosystem and microbial activity is maintained. They also play an important role in stabilizing the soil aggregates. But now plants are being affected by the microorganisms like fungi, present in the soil. Human health, yield of crops and growth of plants are affected by fungal and bacterial diseases. So, to restrict the disease of plants due to fungi, nanoparticles have been used. Nano fungicides based on green chemistry that are ecofriendly and less expensive have been discussed. Environment friendly fungicides cytokinin's, phytohormones and Auxins that could remove fungicides and increase growth of plants. Mechanisms under which fungicides remove have been introduced like destruction of cell membrane and cell wall and biofilm restriction. Synthesis of nanoparticles by chemical and green method and moreover, characterization through different analytical techniques like electron microscopy, X-ray diffraction method and Scanning Electron Microscopy also discussed. The main aim of reviewing is to use metal-based nanoparticles to control the disease of bacteria and fungi, their way of working and possible danger to ecosystem and human being as well. Future outcomes also discussed that reduces harmful fungicides and increase the efficiency of vegetation.

Keywords: Nano Fungicides, plant growth, crop improvement, eco-friendly.

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1. Introduction

New techniques have been introduced for agriculture, which is the main human activity. Due to fungal disease, food quality and yield of crops have been decreased and high dose of fungicides sprayed on crops like fruits, grains and vegetables to reduce the growth of fungi [1]. Additionally, it has been observed that use of fungicides is increased during previous ten years and is repeatedly used in packaging facilities and protected forest areas. About 17.5% pesticides is being applied on crops and 400,000 tons fungicides used all over the world [2]. When fungicides sprayed on crops it enters into agriculture water due to poor management system and it easily diffuses through leaching and subsurface drainage [1]. A lot of studies have done on use of chemical fungicides mainly organic fungicides that effect the human health as well as aquatic or terrestrial organisms it was observed in rat endocrine system and hematological system [3]. For the formation of fungicides, many things like commercial farming, disease, resistance control, market demand and human safety resist companies to spend a lot of money. That is why formulation of fungicides always remains continuous. Most important factors like lasting activity, developmental hazards, and less resistance are characteristics to add in new fungicides formulations. Some fungicides also effect positively on physiological properties of plants [4]. Organic fungicide as alternative fungicides have a lot of benefits including decreasing chance of fungicide resistance and increase effectiveness. Disease control management system has improved due to nanotechnology. These nanoparticles have a lot of benefits including pathogen target selectivity, increased efficiency, reducing environmental hazards and decreased amount of active ingredients [5].

However, Cleanup methods and environmental behavior of nano fungicides has not been discussed widely but nano fungicides have harmful impact on non-target organisms [6]. Another alternative advancement in field of agriculture is chiral fungicides for disease control. Research gap in environmental hazards also mentioned by US environmental protection agency and European commission [7]. Another modern approach is plant antifungal, that mainly depends on immunity activation of plants rather than targeting the fungi [8]. One more non chemical, environment friendly way to control disease of plants that remove many negative effects, is the use of bio fungicides [9]. In the plant protection, during previous years potential of nanotechnology has been explored to control pathogen infesting plants and research on application of nanomaterial is increasing rapidly [10]. Reactivity and surface area of nanoparticles is higher than bulk material in range of 100 nm in one or more dimensions [11-12]. Nanoparticles used in industries as well as biomedicine, possess special properties such as surface effect , small size effect etc [13-14]. Especially metal and metal oxide nanoparticles due to antibacterial properties, are used for plant protection. In this article, we will review existing studies on the use of metal-based nanoparticles to control the disease of bacteria and fungi, their way of working and possible danger to ecosystem and human being as well.

2. Mechanism of action

Nanoparticles decrease the growth and reproduction of both bacteria and fungi including restriction of biofilm formation, destruction of cell wall and cell membrane.

2.1. Destruction of cell wall

The protective covering that is cell wall; is important for growth, replication of bacteria and fungi and also maintain their shape. Therefore for various antibiotics it acts as receptor [15-16]. The major structural component is peptidoglycan in cell walls of bacteria. Meshwork framework that surrounds the cell are made up of disaccharides repeating units [17]. Nanoparticles mainly depend on size, so that they easily enter into cell walls and destroy the internal structure. Various nanoparticles including silver, zinc, iron nanoparticles have been investigated through in vitro experiments and transmission electron microscopy. Previous studies show that nanoparticles did not affect the cell wall of bacteria, they just destroy the cell structure of pathogenic bacteria. But later, it was studied that nanoparticles not only affect the cell wall of microorganisms like bacteria but also disturb the normal biological functioning. As a result death of bacteria occurs [18].

2.2. Destruction of cell membrane

Cytoplasm is protected by a covering that is cell membrane. The most complicated part of the cell. Lipids like glycerophospholipids, sphingolipids, and sterols are the main constituent of cell membrane of fungi. Just like fungi cell, membrane of bacteria is made of phospholipid and protein to some extent [19-20]. The adsorption of nanoparticles on cell membrane is increased due to electrostatic effect that causes destruction of cell membrane. Chen et al. noted through SEM and energy dispersive spectrometry that the zeta potential of the cell membrane is changed due to magnesium oxide nanoparticles [21].

2.2.1. Restriction of biofilm formation

Biofilm is a protecting layer that most bacteria formed to defend themselves from harsh environment. It makes bacteria a highly resistive against antibiotics and protect immune system as well [22]. Magneto responsive gallium based liquid metal nanomaterial will change shape of biofilm under low intensity magnetic field that cause destruction of biofilms and as result bacteria died [23]. Previous studies have suggested that silver , iron and zinc nanoparticles restrict the biofilm in a lot of plant diseases like leaf blight, tomato bacterial wilt and rice brown stripe [24].

2.2.2. Interaction with biomolecules

It has been proposed that morphological features and purpose of biomolecules are affected by nanoparticles when nanoparticles are associated with biomolecules. It was shown by both in vitro and in vivo experiments that DNA is destroyed when titanium oxide nanoparticles in contact with sunlight [25]. Moreover, number of genes that encodes specific enzymes is affected when titanium oxide nanoparticles associated to glycolytic process like NADH (1,4-dihydro nicotinamide adenine dinucleotide) and ATP (adenosine 5'-tri phosphate) in microorganism's in artificial wetlands, formation is decreased (Figure 1) [26].

2.3. Synthesis of Copper nanoparticles

Analytical grade chemicals were used 2.5 g of sodium citrate tribasic dihydrate was dissolved in 100 ml of distilled water and then 5 g of copper sulphate was added. Now added 50ml solution of ascorbic acid about 0.2 ml and 30ml sodium hydroxide solution with continuous stirring, the whole mixture was heated at 95 for about 90 min precipitates black in color obtained which indicate the presence of copper nanoparticles. Precipitates were washed many times using centrifugation method. Dry powder was obtained. X-ray diffraction method like Bruker X-ray diffractometer (processing in Bragg-Brentano geometry and equipped with Cu-anode X-ray source (Ka, l ¼ 0.15418 nm)), is used for characterization of crystal structure. Antifungal activity on mycelial growth was estimated against Fusarium Solani and Fusarium oxysporum. Fungal samples were incubated in PDA solid media. It was mixed with different concentrations of copper nanoparticles. End results were 0.1, 0.25, 0.5, 0.75. Suspensions cells introduced at middle of PDA solid [27]. Copper nanoparticles used as control factor photographic results calculated and percentage measured by formula:

$$\text{IRG (\%)} = \left[\frac{R_1 - R_2}{R_1}\right] \times 100$$

2.4. Zinc oxide nanoparticles

Zinc oxide nanoparticles obtained by dissolving zinc acetate dihydrate in ethylene glycol to get 0.3 M. Solution became transparent by stirring for 20 min. pH reached to 4. Sodium hydroxide is added until pH reaches 9.5, a highly basic solution is obtained. Resulting solution dried for 6 h at about 300 brown colored solid is obtained after maceration, dried solid powder is obtained. Zinc nanohybrids also obtained by extract of garlic and zinc acetate dihydrate used as precursor nitric acid is added to get pH 4, now for added ammonium hydroxide, pH reached to 8.5. Thick solution obtained, filtered and dried. This method is green synthesized method. Different analytical techniques like UV-vis spectroscopy, XRD and Electron microscopy are used for the resulting mixture. Nanohybrids are applied on Mycenae citricolor. 2 cultured were taken, one with nanohybrids and other without fungicide. After 10 days, it showed a significant decrease in growth of fungus [28].

2.5. Synthesis of silver nanoparticles

Seeds of olive plant dried by exposing them to sun. 5 g of dried seeds was boiled in 100 ml flask for 5 min and then it was filtered through Whatman no 1. To get 0.6 μm sized filtrate, mixture is again filtered. Add silver nitrate added to olive extract. 10 ml seed oil and 90 ml silver nitrate were taken so that Ag^+ reduced to Ag and left for 24 h. Because of excitement, reddish brown color indicates the presence of silver nanoparticles. To check antifungal activity silver nanoparticles was applied to *Aspergillus niger*, *Sclerotium aolfsii* and *Alternaria macrospora*. By pore plate technique fungal spore was mixed with agar plate and 3 cavities were formed by using the Czapek Dox plate. Plates were kept at room temperature for 7 h. Clear zone was formed indicate the inhibited growth of fungus (Figure 2) [29].

2.5.1. Comparative evaluation

Antifungal capacity of zinc oxide nanoparticles has been explained by using chemicals route and secondly by green synthesis route for the formation of zinc oxide nanoparticles in an extract of garlic (*Allium sativum*).IR spectroscopy Xray, UV-visible, SEM and TEM used. It was concluded that green synthesis route is best because it produces nanometric size particles which have larger surface area to volume ratio. This method best for both Colletotrichum's and Citricolor as it inhibits the growth of fungi so due to nanoparticle size that allows them to pass easily through cell membrane cause cellular dysfunction of fungi and produces reactive oxygen which effect protein of cell wall [28]. Synthesis of fungicides that are ecofriendly and cost effective on principle of green chemistry have been explained. Copper nanoparticles were synthesized for plant disease due to fungi. Antibacterial activity is different for different fungi so copper fungicides proved strong against *Oxysporum* sp. and solani because it damages directly to cell membrane of plant pathogens and is much more effective against plant pathogens. XRD and TEM used for analysis [27]. As olive seeds contain biological chemicals. It was observed that silver nanoparticles can be made from olive seeds had more antimicrobial effect. FTIR and TEM used. DNA synthesis effected by silver. The main role is to kill pathogenic fungi. Sensitivity of silver nanoparticles observed for *Aspergillus niger* have restriction zone 1.6 cm while for *Rhizoctonia batali* cola was 1.3 cm [29]. Copper fungicides work more effectively than other fungicides due to high antimicrobial activity and environment friendly (Table 1).

2.6. Hazards of chemical fungicides and effect on environment

2.6.1. Inorganic fungicide

Copper and Sulphur are most used inorganic fungicide to manage fruit diseases like foliar fruit diseases. European Union have been approved the copper and Sulphur for organic observation. Inorganic fungicides adsorbed in soil and flow through surface water in response to biogeochemical cycle [30]. Cu^{2+} releases as initial oxidation ion when copper oxide dissolved in water [31]. Bivalves and barnacle act as water dwelling insects while fish, algae and decapod crustaceans accumulate the copper moreover cyanobacteria are sensitive to copper and diatoms resistive to copper. As the previous studies suggested that copper cannot be broken down in soil, it aggregates in soil and effects the overall yield of crops and concentration of copper in soil is from moderate to low. It was investigated that high level of copper in soil effect the crop quality and overall yield is decreased [32]. Presence of copper in soil is badly influenced the various processes like resistance to pathogens, biodegradation and overall morphology of soil and microorganism [33]. Copper also reduces the growth of environment friendly bacteria and fungi [34]. Due to extremely high concentration of copper, normal activity of microorganisms can be reduced according to Diaz Ravina et al. For example, arbuscular mycorrhiza fungi (AMF) is highly effected by copper fungicide [35]. Another inorganic fungicide is Sulphur mainly in form of (e.g., SO_3) and salt (e.g., $MgSO_4$) in environment. Sulphur is present in soil and sediments in very low amounts as a micronutrient. It reduces the growth of environment friendly fungi and bacteria and also effect some plants like apricot, cucurbits and raspberries [36-37]. Kuklinska et al. [28] also indicated that Sulphur effect the Vibrio fishery as well. Previous data suggested that Sulphur is not much dangerous as copper [34].

2.6.2. Organic fungicides

When organic fungicides are used in high concentration it affects the plants and leads to water pollution in various crops like vineyard, horticulture, orchards. Two types of fungicides were discussed. In growing season, curative fungicides were observed and in early season, preventive fungicides were observed. Previously studied that fungicides remain in ground water and pollute it [38]. New establishment was taken for a lot of fungicides because of secondary side effects. Amistar and Monarch studied the effect of azoxystrobin and flutolanil on potato plants disease AMF rhizophagus irregularis MUCL 41,833 and doses used to manage Rhizoctonia solani and impact of processes like spore germination and radial development. It was observed that spore germination and extra radial development is decreased by azoxystrobin and flutolanil did not effect. Flutolanil effected the root colonization and arbuscule formation [39].

2.6.3. Chiral Fungicides

Many fungicides have asymmetric centers like enantiomers and Di stereoisomers. Di stereoisomers have different properties and activities while enantiomers have different chiral environments but same physiochemical properties. Previously discussed that Half-life is the main factor that shows the behavior of chiral fungicides. Different fungicides have different half-life like enantiomeric fungicides. When pencazole was studied in field it was observed that negative enantiomeric fungicides breakdown more rapidly than positive fungicides [40].

2.6.4. Nano fungicides

Nanoparticles like zinc oxide nanoparticles, silver oxide and copper oxide nanoparticles. It protects the plants from harmful fungi, but it has some disadvantages as well it effects the properties like surface charge, cation species and soil sorption capacity and as a result a huge change in toxicity level occurred. Copper oxide and zinc oxide nanoparticles are less toxic and beneficial for plants. Actually, nanoparticles get into plants through cell membrane by endocytosis, pore formation and carrier proteins. Most of the plants have defense systems but sometime this system fails to work and overall growth of plants is affected.

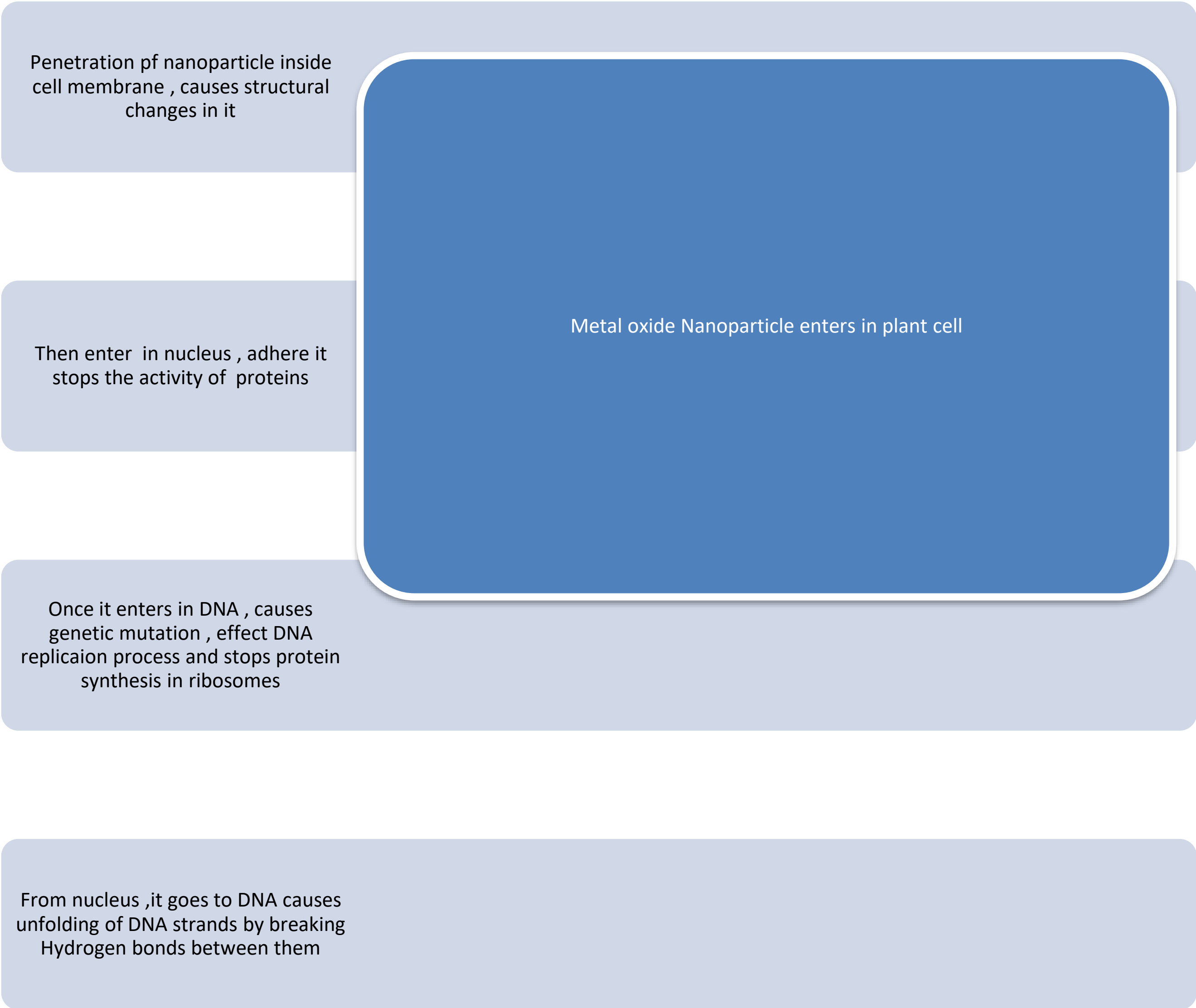

Figure 1. Interaction with biomolecules

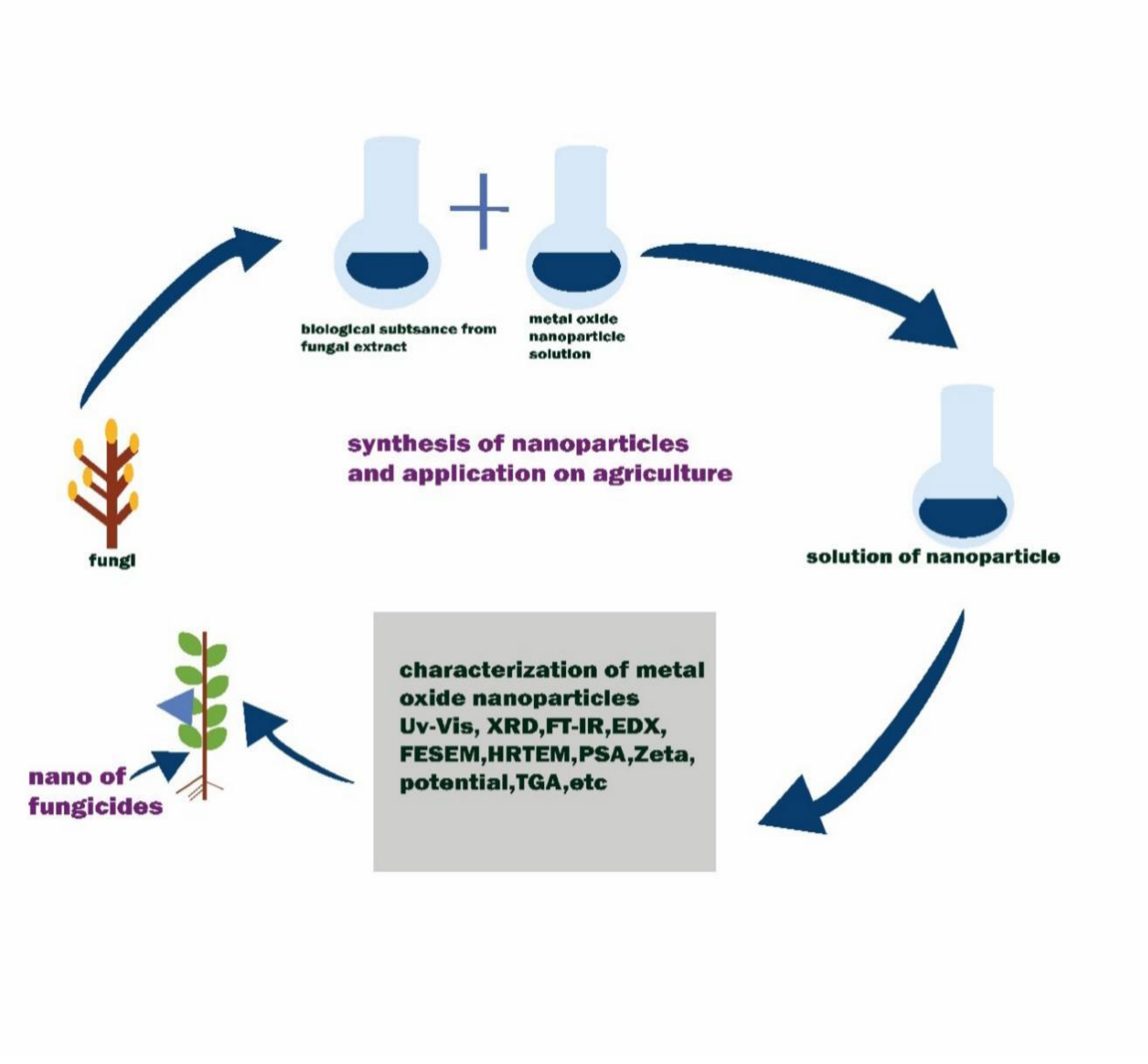

Figure 2. Schematic representation of biosynthesis of Nano fungicides from fungal culture [44]

Table 1. Effect of metal-based nanoparticles on fungi and infected plants

Nanoparticles	plant	Causative agent	Consequences	References
Silver biosynthesis	tomato	Fusarium graminearum	Restriction of mycelial growth and germ tube length	[45]
Copper	chrysanthemum	Fusarium oxysporum	High antimicrobial activity, disease severity rate decreases by 32% in recent years	[46]
Zinc	tomato	Fusarium solani	Effect spore germination and mycelial growth, no effect on fresh weight and dry weight of infected plants	[47]
Silver	tomato	Alternaria solani	Increase of chlorophyll and total weight content, decrease proline content	[48]

For example, it was observed there is a decrease in amount of chlorophyll a and b when nanoparticles in contact with maize plant [41].

2.7. Strategies for Fungicide accumulation Improvement

2.7.1. Plant growth regulators and phytohormones

Small organic molecules responsible for growth , development , immunity of plant and controlling the biotic and abiotic response are phytohormones [42] Cytokinin's CK, salicylates SA, ethylene ET and jasmonates JA are the major phytohormones, act as defense regulators for a lot of plants pathogens like fungi [43]. Efficiency of CW to remove fungicide increased by Melatonin. The expression of many genes is also controlled by melatonin. It also increases the positive effects on plants. It has been suggested that melatonin is important for management of osmosis and both primary and secondary metabolism.

Also, Nitrogen species like hydrogen peroxide, nitric oxide and hypochlorous acid and reactive nitrogen balanced by melatonin and these are hazardous for plants [49]. According to Rostami et al. the overall biomass of roots and stems is increased by auxin. There is an increase in release of exudates from root of plants to rhizosphere which results in greater growth of root system. Root system creates conditions that increase the growth of population and activity of microorganisms. Growth conditions , genus and species of bacteria depends on quantity of auxins [50]. The major role of cytokinin's is the synthesis of chlorophyll. It converts ethioplast to chloroplast by using free radicals and leaves look young due to presence of cytokinin. Consequently, Plants are able to change themselves according to environmental conditions. Studies suggested that production of glutathione is increased by cytokinin while interacting with plant defense systems. plant biomass is increased by cytokinin [51].

2.7.2. Chelation

EDTA ($EDTANa_2$) serves as a chelating agent that improves the absorption of micronutrients in agricultural contexts. 90% Fusarium graminearum in wheat and growth of fungi is controlled by EDTA. While it enhances the availability of nutrients and soil pH, careful dosing is necessary because it can reduce growth and raise environmental concerns [52]. Phytoremediation system is studied for chelates. Researchers have a look on more environment friendly chelating agents. The increased efficiency of amaranth (*Amaranthus hypochondriacus* L). Admium contaminated soil by chelators like glutamic acid, nitrilotriacetic acid, citric acid and ethylene diamine Di succinic acid. In start plants restrict the chelators but use of glutamic acid help to increase the overall yield of plants, also the activity of enzymes in soil was increased twice when both glutamic acid and nitriloacetic acid used [53].

3. Conclusion and Future outcomes

The most important tool used to protect the plants is fungicides. Nowadays advancements introduce new environmentally friendly fungicides like nano fungicides. Nano fungicides play an important role in reducing the growth of fungi are copper oxide, silver oxide and zinc oxides. These particles enter in plants through cell membrane by endocytosis or pore formation and destroy the biofilm of fungi and morphological features. Excess of everything is bad so if nano fungicides are used in high amount, it will affect the plants. Among all the copper and zinc nanoparticles are less hazardous to plants. Auxins and cytokinin's are growth hormones that increase overall biomass of roots and chlorophyll content of plants. Environment is highly affected by chemical fungicides like organic, inorganic and chiral fungicides, so there is a need to use new way to kill pathogens in plants without effecting both nature and environment as well. Proteomics have been used with high rate of success e.g., formation of new chemical fungicides. it could be used together with environmentally safe scheme to kill fungi in plants.

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