

Review

Nanoparticles as Potential Antivirals in Agriculture

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Abstract: Viruses are estimated to be responsible for approximately 50% of the emerging plant diseases, which are difficult to control, and in some cases, there is no cure. It is essential to develop therapy practices to strengthen the management of these diseases caused by viruses in economically important crops. Metal nanoparticles (MeNPs) possess diverse physicochemical properties that allow for them to have a wide range of applications in industry, including nanomedicine and nano-agriculture. Currently, there are reports of favorable effects of the use of nanoparticles, such as antibacterial, antifungal, and antiviral effects, in animals and plants. The potential antiviral property of MeNPs makes them a powerful option for controlling these histological agents. It is crucial to determine the dosage of NPs, the application intervals, their effect as a biostimulant, and the clarification of the mechanisms of action, which are not fully understood. Therefore, this review focuses on discussing the ability of metal nanoparticles and metal oxides to control viruses that affect agriculture through an exhaustive analysis of the characteristics of the particles and their interaction processes for a possibly beneficial effect on plants.

Keywords: plant virus; diseases; nanotechnology; biostimulant

1. Introduction

Nanoparticles (NPs) are generally defined as particulate matter with sizes ranging from 1 to 100 nm [1]. This broad class of materials can be classified into metallic and non-metallic nanoparticles; the latter consists of ceramic nanoparticles (e.g., hydroxyapatite, silica, alumina), polymeric nanoparticles, and solid lipid nanoparticles [2]. The importance of nanoscale compounds is derived from the inherent properties they possess (optically active, reactivity toughness, surface area, mechanically strong, and chemically reactive). NPs offer improved features depending on their size, shape, and structure, some of which are not present in bulk materials [1]. Although nanoparticles allow for a wide range of applications in areas including catalysis, photonics, molecular computing, energy storage, fuel cells, and tunable resonant devices [3], NPs have received much attention from some scientists and researchers in different areas of the biological sciences [4]. Nanobiotechnology is biotechnology at the nanoscale and covers many applications in other fields, from agriculture to medicine [5]. Nanoscale particles have been explored in various biological applications such as biosensors, biological separation, molecular imaging, cancer therapy [6].

Nanomedicine, for its part, allows for the diagnosis, treatment, and prevention of diseases [7] due to NPs' antimicrobial, antiviral [8,9], and anticancer [10] properties, among others. The successful use of nanoparticles in the area of *in vitro* nanomedicine has generated considerable interest in their application in the field of agro-nanotechnology [11]. The advantages of its use in crop production are a reduction in the use of chemicals, water, and nutrient management; improving the assimilation during fertilization; increased yield through pest and nutrient management [12].

Plant viruses have a severe negative impact on agriculture and it is essential to make considerable efforts to control viral diseases [13]. According to one estimate, viruses are responsible for approximately 50% of the emerging plant diseases, and agriculture has a vital role in their dispersion [14]. Almost 900 species of plant viruses that infect more than 700 crop species have been reported [9,15]. The magnitude of the impact of viruses on plants varies depending on the stage of infection, environment, strain of the virus, type of host, speed of replication, and virus concentration [16]. Under favorable circumstances for the pathogen, the symptoms that viral infections can cause are mottling, drying of leaves, malformation of fruit, necrosis, plant stunting, and even death [9]. The main problem in the control of diseases is that they are challenging to cure, and in the case of viruses, there is no cure; the only options are to prevent or avoid infection [17,18]. Currently, nanoscale materials have emerged as novel antibacterial, antifungal, and antiviral compounds for use in animals and, to a lesser extent, in plants [9,19,20]. The development of antiviral agents is a promising area and the use of metal nanoparticles can be a new opportunity for novel antiviral therapies in crops. As such, in this study, we analyzed the potential of metal nanoparticles as a therapy against pathogenic viruses that damage plants, creating areas of opportunity for the use of metal nanoparticles for the management of viral diseases in agriculture.

2. Current Options for Controlling Virus Diseases in Agriculture

The worldwide loss of cultivation caused by viruses reaches around 20%. However, some infections can generate 100% crop losses, mainly in developing countries, reaching a cost of USD 60 billion annually [9]. The problems caused by the virus have been the aggravation of crops due to changes in cultural practices and climate [21]. Virus infections have become significant and demand effective management practices to control them. Some strategies carried out in greenhouses are the reduction of insect vector populations through the use of chemical compounds or biological control [22]. Mainly because viruses depend on insect vectors for their survival, transmission, and propagation [23]. Other practices include the reduction of virus sources (infected plants or plant remains) interference with a vector's landing by altering the attraction of insects to colors, and interference with the transmission process through the use of mineral oils [22].

Other techniques that have been carried out in the laboratory for the management of viral diseases in crops include conventional and advanced practices [15]. Within conventional strategies, we find meristem tip culture, cryotherapy, thermotherapy, and chemotherapy. However, these techniques have many limitations since they are only used in tissue cultures and consider the reduction of the initial inoculum. In comparison, the advanced methods consist of RNA silencing, cross-protection, transgenic plants, gene pyramiding, and protein–protein interaction [15]. In the latter, the virus can be eliminated in plant tissues (as opposed to simply avoiding or delaying the infection), though this process does interfere with the vectors, use of attenuated strains and resistant or tolerant varieties, and is very expensive [24–26]. Chemotherapy consists of the use of antivirals that treat some diseases in animals and is also useful for the treatment of viruses in plant tissue cultures [27]. Antivirals used in plant tissue culture conditions are acycloguanosine (Acyclovir), 5-azacytidine, cytarabine, 5-bromouracil, ribavirin (Virazole), 2-thiouracil azidothymidine (Zidovudine), and 2, 4-dioxohexahydro-2, 5-triazine (DHT). Some treated diseases are potato leafroll virus (PLRV) [28], grapevine vitivirus [29], sugarcane mosaic virus (SCMV) [30], and Indian citrus ringspot virus (ICRSV) [27]. The disadvantages of chemical antivirals are that they all have different modes of action, they are not effective *ex vitro*, and none are selective enough to be useful against viral crop diseases [15].

Meanwhile, advanced methods, such as the RNA interference (RNAi)-mediated response, consist of silencing through dsRNA. The excess RNA is modified to dsRNA using RdRP (RNA-dependent RNA polymerase), allowing for RNA silencing; this technique is also used to control vector insects. However, studies are necessary for the evaluation of resistance efficiency in the crop field [15]. The control of viral diseases in plants also includes the use of resistant varieties or a cross-protection technique, which consists of a systematic infection with a second virus to induce resistance to the target virus. One of the major approaches undertaken to reduce crop losses is the use of resistant varieties since they are the most economical and most useful; however, the development of highly effective and durable virus-resistant/immune crop varieties is required [31]. Furthermore, gene pyramiding involves the stacking of multiple genes, resulting in the simultaneous expression of various genes in a variety; however, its disadvantages are its epistatic effect and high costs [15].

3. Metallic Nanoparticles

Metallic nanoparticles (MeNPs) comprise pure metals and metal oxides, where the latter are produced by the addition of oxidizing agents (e.g., H_2SO_4 , KMnO_4 , or $\text{K}_2\text{Cr}_2\text{O}_7$) [32]. Some examples of the most common MeNPs include gold (Au), nickel (Ni), silver (Ag), platinum (Pt), and iron (II, III). On the other hand, the metal oxide nanoparticles (MoNPs) include several compounds, such as Al_2O_3 , CeO_2 , CuO , Cu_2O , In_2O_3 , La_2O_3 , MgO , NiO , TiO_2 , SnO_2 , ZnO , and ZrO_2 [33]. The morphological characteristics of the nanoparticles depend on the conditions of the processes used to make them. The production of nanoparticles is done using two basic strategies: top-down, which consists of breaking the material, while the bottom-up strategy creates nanomaterials from atoms or molecules [34]. The top-down strategy includes mechanosynthetic, compaction and consolidation, thermal, high-energy, plasma, chemical fabrication, and lithographic methods. On the other hand, bottom-up approaches include chemical vapor deposition, atomic layer deposition, molecular beam epitaxy, and liquid-phase methods. Chemical, physical, or biological methods can produce nanoparticles. The advantage of physical processes is that it is possible to obtain more significant quantities of nanoparticles. In contrast, the colloidal chemical synthetic process can control the size and shape of the nanoparticle [35]. There are also green nanoparticle synthesis methods that are used to reduce the damage caused by various chemical compounds [36]. The objective of green synthesis is partly to decrease the use of dangerous reagents and products and to save energy [37]. Compounds present in the plant extracts that are used to carry out the production of nanoparticles are ketones, aldehydes, flavones, amides, terpenoids, carboxylic acids, phenols, and ascorbic acid [38]. However, the problems faced by the different synthesis methods for these types of compounds are the functionality of the NPs and control of the crystal growth, stability, and mobility, as well as controlling the agglomeration, size, and shape [39]. It is essential to control several additional physicochemical variables (pH, ionic strength, the concentration of NPs, and natural organic matter) to avoid these problems [40].

Because the synthesis methods largely determine the geometry of the nanoparticles, these affect the physicochemical properties of NPs, such as the size, dispersity, type of extrinsic and/or intrinsic defects, morphology, and crystal structure [3]. The size of the NPs influences their entry into cells and interactions with biomolecules [41]. On the other hand, the cellular uptake efficiency of nanostructures is dependent on their size, surface charge, and shape [42]. The diversity of the characteristics of the metal nanoparticles relates to their toxicity. Some authors believe that the toxicity of metallic nanoparticles is related to the production of reactive oxygen species (ROS), which depends on the particle size, shape, surface area, and chemistry [43]. Some studies have reported the creation of ROS as O_2 in TiO_2 , ZnO , V_2O_5 , CeO_2 , Fe_2O_3 , and Al_2O_3 under photoillumination [44]. Thus, the toxicity of MeNPs not only affects humans and the environment, the toxicity is also produced in microorganisms and can influence the antibiotic, biocidal, antifungal, and antiviral activity [43]. As such, MeNPs are proposed as a promising antiviral agent.

4. Antiviral Activity of Metallic Nanoparticles

Viruses are submicroscopic particles made out of RNA or DNA and a capsid composed of proteins. They only multiply in the host cells at the expense of the cells' metabolism [45]. The virus multiplication cycle involves several events: the entry into the cells, the synthesis of the genome and viral proteins, the assembly of viral components, and the viral output of infected cells [46]. Consequently, if a compound can inhibit one or more stages of the virus cycle, it can be considered an antiviral agent [46]. Due to the ability of MeNPs to intervene in some steps of virus replication, they have been studied for antiviral therapy in recent decades [47]. The use of nanoparticles against viruses focuses on the detection or treatment of diseases, such as the human immunodeficiency virus, hepatitis A, B, and C (HAV, HBV, HCV), influenza virus, and herpes virus 1 and 2 (HSV-1, HSV-2) [48]. Studies have been developed along two lines: the antiviral activity of (1) functionalized MeNPs or (2) pure or non-functionalized nanoparticles [47]. Regarding the latter, the main compounds evaluated are gold and silver, as well as some metal oxide nanoparticles, such as iron oxide, zinc oxide, and titanium dioxide. Notably, several studies have demonstrated the antiviral activity of metal nanoparticles against human viruses. It seems that silver nanoparticles display a broad spectrum of antiviral activity against viral infections from the Retroviridae, Herpesviridae, Paramyxoviridae, Poxviridae, Orthomyxoviridae, Arenaviridae, and Hepadnaviridae families [48]. Within the most important studies, AgNPs have shown antiviral activity against influenza, foot-and-mouth disease virus, HIV-1, herpes simplex, hepatitis B, HSV-1, respiratory syncytial virus, H1N1, and H5N1 influenza A virus [49–52]. The mechanisms of action used by silver nanoparticles against viruses are the interaction with gp120, competition for the binding of the virus to the cell, inactivation of the virus particles before entry, interaction with double-stranded DNA, and binding with viral particles [48]. Gold/copper sulfide core-shell nanoparticles (Au/CuSNPs) induce viral capsid protein degradation and capsid damage [53]. Furthermore, the antiviral effect of metallic nanoparticles is due to a synergistic effect with other chemical compounds. Selenium nanoparticles (SeNPs) enhance the anti-EV71 activity of oseltamivir in the human astrocytoma cell model [54].

In the case of metal oxide nanoparticles, Levina et al. [55] reported that TiO₂-polylysine (PL)-DNA nanocomposites could be successfully used for the highly efficient and site-specific inhibition of influenza A viruses of different subtypes. Cui et al. [56] reported the inactivation of H9N2 avian influenza virus in cells exposed to an anatase nano-TiO₂ solution under UV irradiation at 365 nm. The above suggests that MeNPs under photoillumination induce the production of radicals, which makes them toxic. However, Mazurkova et al. [57] reported that TiO₂ nanoparticles inactivate the influenza virus independent of illumination with light, which indicates that antiviral activity is not related to photocatalytic effects. The inactivation of the virus likely occurs due to the union and destruction of the virus envelope by nanoparticles. In contrast, *in silico* studies of the interaction of TiO₂ nanoparticles with twenty amino acids in aqueous solution indicate that charged amino acids play a dominant role during the process of bonding to the TiO₂ surface, with basic and acidic residues being preferred over those that are not loaded. When calculating the mean potential strength, the amino acid Arg is prone to direct bonding on the surface of the NPs. In contrast, Lys needs to overcome a free energy barrier of ~2 kT. Moreover, acid residues tend to form hydrogen bonds between their side chains and the TiO₂ surface, thus showing an indirect link [58].

In the case of the HSV-1 virus, partially negatively charged ZnO nanoparticles effectively trap virions through a novel virostatic mechanism that renders them incapable of entering human corneal fibroblasts [59]. Tavakoli et al. [60] determined the inhibitory effects of zinc oxide nanoparticles (ZnONPs) and polyethylene glycol (PEG)-coated ZnONPs (ZnO-PEG-NPs) on HSV-1. The results indicated that some changes in the nanoparticles, such as the PEGylation of ZnO nanoparticles, decrease the cytotoxicity and increase the antiviral activity against HSV-1. On the other hand, ZnO nanoparticles interact with the Chikungunya virus, showing an antiviral effect. Other metal oxide nanoparticles, such as tin oxide (SnO₂) nanowires, have demonstrated antiviral activity as an effective treatment

against HSV-1 infection, where these nanoparticles carry negatively charged structures that compete with the HSV-1 attachment to cell-bound heparan sulfate (HS), avoiding cell spread [34].

5. Antiviral Activity of MeNPs in Plants

Plant viruses are a challenge for the production of vegetables. Studies have focused on the use of nanoparticles for the management of plant diseases; however, research aimed at combatting viral diseases is still in its beginning stages. The antiviral mechanisms of MeNPs are not well understood but current available studies could provide evidence of the mechanisms involved. The antiviral activity of MeNPs has been demonstrated both *in vitro* and *in vivo* with different plants and is effective against positive- and negative-sense single-stranded RNA viruses (Table 1). Many authors describe the interaction of MeNPs with the surface of the virus as one step of the antiviral mechanism. Cai et al. [9] reported on the antiviral activity of ZnONPs and SiO₂NPs against tobacco mosaic virus (TMV) *in vitro*. The results of this work suggest a direct inactivation of TMV by MeNPs due to the interaction with envelope glycoproteins, which causes the direct injury of TMV shell proteins, TMV aggregation, and even fracture. TMV particles treated with Fe₃O₄NPs display the same aggregation and fracture; furthermore, direct interactions between Fe₃O₄NPs and TMV particles lead to larger particle sizes [61]. AgNPs can also bind to coat protein virus particles of the tomato mosaic virus (ToMV) and potato virus Y (PVY) [62]. Meanwhile, Alkubaisi and Aref [63] showed that AuNPs cause damage to the virus-like particles (VLPs) of the barley yellow dwarf virus-PAV, where they observed puffed and deteriorated VLPs decorated with AuNPs, as well as destroyed and vanished particles, using TEM.

Table 1. Antiviral activity of metal and metal and metal oxide nanoparticles.

Nanoparticle	Size	Plant	Pathogen	Effect	References
Silver nanoparticles (AgNPs)	10–20 nm	<i>Cymopsis tetragonaloba</i>	Sunhemp rosette virus (SHRV)	Complete suppression of the disease	[64]
AgNPs	77 nm	<i>Vicia faba</i>	Bean yellow mosaic virus (BYMV)	Decrease in virus concentration, percentage of infection, and disease severity Reduction in lesions on infected leaves	[65]
Schiff base nanosilver NPs		<i>Nicotiana tabacum</i>	Tobacco mosaic virus (TMV)	Reduction of the harm of TMV to tobacco	[66]
AgNPs	12 nm	<i>Solanum tuberosum</i>	Potato virus Y (PVY)	Resistance to virus infection	[67]
Graphene oxide-silver NPs (GO-AgNPs)	3050 nm	<i>Lactuca sativa</i>	Tomato bushy stunt virus (TBSV)	Decrease in virus concentration, infection percentage, and disease severity	[68]
AgNPs	12.6 ± 5 nm	<i>Solanum tuberosum</i> L. cv. Spunta	Tomato spotted wilt virus (TSWV)	Decrease in TSWV infectivity and produces an inhibitory effect in local lesions	[69]
AgNPs	-	<i>Solanum lycopersicum</i>	Tomato mosaic virus (ToMV)	Reduction in disease severity and virus infection	[62]
AgNPs	-	<i>Solanum lycopersicum</i>	Potato virus Y (PVY)	Reduction in disease severity and virus infection	[62]
Gold NPs (AuNPs)		<i>Hordeum vulgare</i>	Barley yellow mosaic virus (BaYMV)	Dissociation of virus particle in vitro	[70]
AuNPs	3.151 to 31.67 nm	<i>Hordeum vulgare</i>	Barley yellow dwarf virus-PAV (BYDV-PAV)	Destroys gold barley yellow dwarf virus and eliminates virus infectivity hazards	[63]
Titanium dioxide NPs (TiO ₂ NPs)	~3–5 μm	<i>Vicia faba</i> L. Fabaceae	Broad bean stain virus (BBSV)	Reduction in disease severity	[71]
TiO ₂ NPs	20 nm	<i>Nicotiana benthamiana</i>	Turnip mosaic virus (TuMV)	Effectively limits viral infection and replication	[72]
Zinc oxide NPs (ZnONPs)	18 nm	<i>Nicotiana benthamiana</i>	Tobacco mosaic virus (TMV)	Suppression of the speed of TMV invasion	[9]

Table 1. Antiviral activity of metal and metal and metal oxide nanoparticles.

Nanoparticle	Size	Plant	Pathogen	Effect	References
ZnONPs	-	<i>Solanum melongena</i>	Cucumber mosaic virus (CMV)	Disease severity and incidence were significantly reduced	[73]
Silicon dioxide NPs (SiO ₂ NPs)	-	<i>Cucumis sativus</i>	Papaya ringspot virus (PRSV)	Reduction in disease severity and virus accumulation	[74]
SiO ₂ NPs	100 nm	<i>Solanum lycopersicum</i>	Tomato yellow leaf curl virus (TYLCV)	Reduced disease severity and TYLCV concentration	[75]
SiO ₂ NPs	20 nm	<i>Nicotiana tabacum</i>	Tobacco mosaic virus (TMV)	Suppression of the speed of TMV invasion	[9]
Iron oxide NPs (Fe ₂ O ₃ NPs)	40–100 nm	<i>Nicotiana tabacum</i>	Turnip mosaic virus (TuMV)	Effectively limits viral infection and replication	[72]
Fe ₃ O ₄ NPs	0.19 nm	<i>Nicotiana benthamiana</i>	Tobacco mosaic virus (TMV)		[9]
Nickel oxide NPs (NiONPs)	15 to 20 nm	<i>Cucumis sativus</i>	Cucumber mosaic virus (CMV)	Reduces disease severity and CMV concentration	[76]
Cerium oxide NPs (CeO ₂ NPs)	-	<i>Datura stramonium</i> and <i>Nicotiana tabacum</i>	Tobacco mosaic virus (TMV)	Reduction in virus symptoms	[77]

In vivo studies of the effectiveness of MeNPs against viral infections in the Solanaceae, Asteraceae, Poaceae, Fabaceae, and Cucurbitaceae families have also been conducted. The application of MeNPs in plants interferes with the reproduction of viruses through different mechanisms. Furthermore, nanoparticles affect the diseases caused by viruses. CeO₂NPs inhibits the reproduction of viruses by penetrating the TMV-infected leaf segment of *Datura stramonium* and *Nicotiana tabacum* through the vascular system and inhibits reproduction [77]. Graphene-based silver nanocomposites exhibit potent activity against tomato bushy stunt virus (TBSV) in lettuce, decreasing the virus concentration and disease severity [68]. The antiviral activity of the NPs was observed in both the foliar application and the soil supplementation. *Cucumis sativus* infected with papaya ringspot virus (PRSV) and treated with SiO₂NPs via soil supplementation produced a significant reduction in disease symptoms [74]. Application of TiO₂NPs using sprays and soil drenches after 24 h of broad bean strain virus (BBSV) inoculation in faba bean plants causes a dramatic decrease in BBSV. The antiviral effect and the responses of the plants depend on the treatment time, that is, whether they are applied before, along with, or after the viral infection. Elbeshehy et al. [65] studied the effect that AgNPs synthesized by the bacteria *Bacillus pumilus*, *Bacillus persicus*, and *Bacillus licheniformis* had on yellow bean mosaic virus. A post-infection treatment 24 h after virus exposition prevented all negative symptoms caused by the virus. However, plants treated with the AgNPs and infected simultaneously showed weak BYMV symptoms, and a pre-infection treatment 72 h before inoculation showed no effect on the virus concentration and disease severity.

Chenopodium amaranticolor plants showed the most significant antiviral effect against tomato spot wilt virus (TSWV) when the AgNPs were sprayed 24 h after inoculation. Plants sprayed after inoculation showed a weak infection, and plants sprayed before inoculation displayed weak inhibition [69]. El-shazly et al. [67] reported a decrease in virus concentration and disease percentage for potato plants sprayed with AgNPs 24 h after inoculation with tomato bushy stunt virus (TBSV) [67]. On the other hand, *Cymopsis tetragonaloba* leaves sprayed with AgNPs and sunhemp rosette virus (SHRV) showed complete suppression of the disease by inactivating virus replication [64]. Other authors presented studies where there was antiviral activity when NPs were applied pre-inoculation. A reduction in the severity of the virus was also reported for cucumber plants treated with nickel oxide NPs and for tomato plants sprayed with silica nanoparticles 1 day before tomato yellow leaf curl virus (TYLCV) inoculation [75]. A mixture of AgNPs and salicylic acid (SA) applied before 3 and 7 days of virus infection shows a synergistic antiviral effect against TBSV compared to individual AgNPs and SA treatments [67]. The above shows the ability of NPs mixed with other compounds to present a synergistic effect. Some authors suggest that MeNPs activate the immune system of plants [9,61,72,76]. To evaluate the protective effect of nanoparticles in tobacco plants (*Nicotiana benthamiana*) contra TMV, Cai et al. [9] applied ZnO and SiO₂ NPs 3, 7, and 12 days before inoculating with the virus; the 12-day treatment exhibited high antiviral effects and more efficiently prevented TMV from spreading to newly emerged leaves. Tobacco pretreated with Fe₂O₃ or TiO₂NPs for 21 days showed antiviral properties, decreased the amount of the viral protein of turnip mosaic virus (TuMV), which the authors suggest could be related to the fact that the NPs interfered with either protein biosynthesis or post-translational modification processes in the virus, and activated defense mechanisms [72]. AgNPs sprayed on infected tomatoes for 7 days before inoculation with ToMV and PVY reduced the disease severity and virus concentration because the NPs were bound to the virus and inhibited virus replication [62]. *Nicotiana benthamiana* leaves pretreated with Fe₃O₄NP for 12 days showed high resistance to TMV, which is even more potent than lentinan. Furthermore, the authors reported that NahG transgenic plants, i.e., plants that degrade SA, did not reduce virus accumulation after 12 days of Fe₃O₄NP treatment. The above indicates the generation of plant resistance induced by Fe₃O₄NPs was mainly due to the upregulation of SA synthesis [61]. Some complexes with metals, for example, chitosan Schiff base nanosilver (S-cos-Ag), significantly reduced the number of lesions in TMV-infected tobacco leaves, inducing TMV resistance and promoting the tobacco's immunity [66]. These studies support the idea of Cai et al. [61], which states that the use of MeNPs as antivirals in plants is due not only

through the inactivation of viruses and inhibition of multiplication but also the activation of plant defense mechanisms that leads to plant immunity and growth response, in which the antioxidant system, resistance genes, and plant hormones participate (Figure 1).

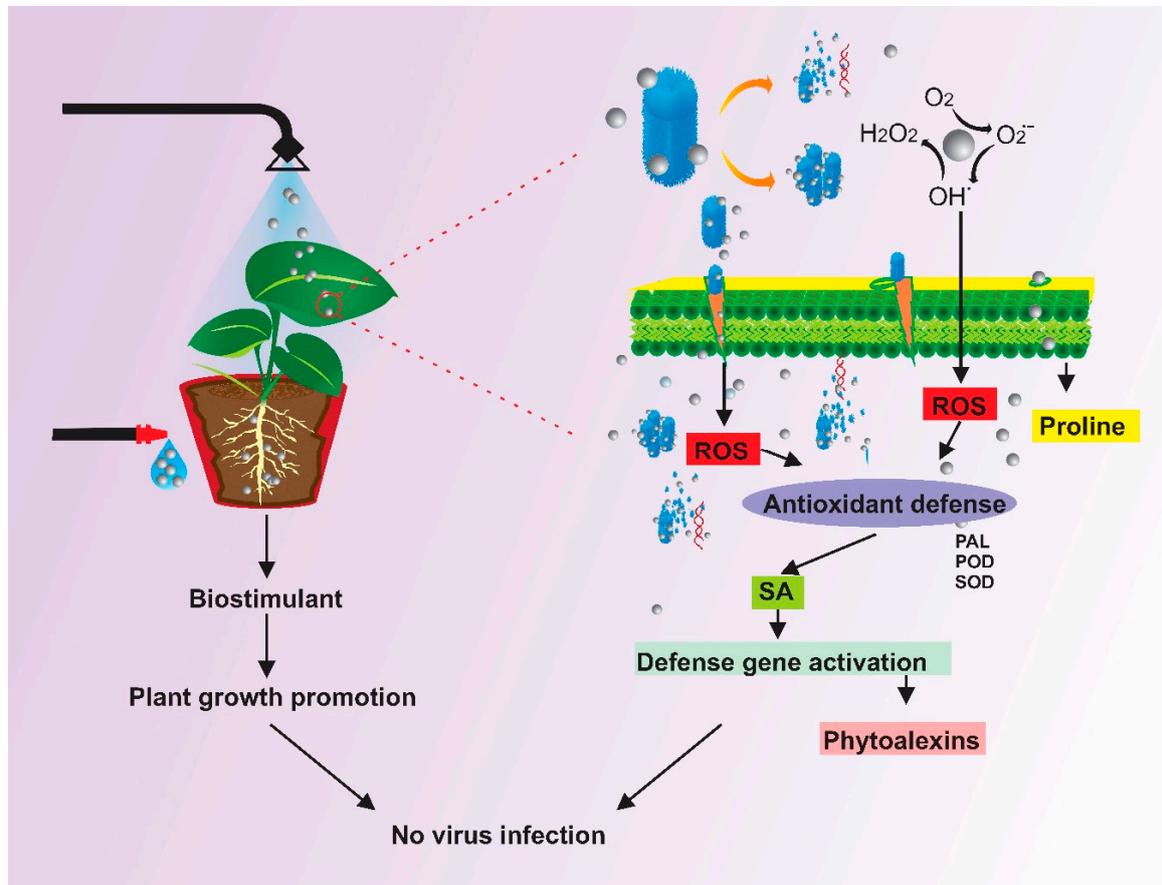


Figure 1. Antiviral mechanism of metal and metal oxide nanoparticles in plants. PAL: phenylalanine ammonia-lyase; POD: peroxidase; ROS: reactive oxygen species; SA: salicylic acid; SOD: superoxide dismutase.

6. Viral Inhibition via Plant Defense Induction Using NPs

6.1. Antioxidant System

The general plant response to stress is the increase in reactive oxygen species (ROS) levels, which limits pathogen ingress and distribution and induces systemic and local defense responses, such as the activation of pathogenesis-related (PR) genes [9]. Many biotic and abiotic stimuli induce ROS production in plants. When ROS levels (O_2 , H_2O_2 , OH , and $1O_2$) are higher than the threshold, they generate oxidative products and the balance between ROS and antioxidants is interrupted. The antioxidant system allows plants to counterbalance the effect of oxidants. Superoxide dismutase (SOD) is the first line of defense and is responsible for the transformation of O_2 into H_2O_2 and water [78,79]. The antioxidant system also consists of enzymes, such as catalase (CAT), ascorbate peroxidase (APX), and guaiacol peroxidase (GPX) [79]. Depending on the type of MeNPs, the concentration, and the type of culture, MeNPs interfere with cellular redox homeostasis by either inducing or reducing the occurrence of oxidative stress [78]. Studies have shown that the application of TiO_2 , CeO_2 , ZnO , CuO , Ag , NiO , Al_2O_3 , $CoFe_2O_4$, Fe_3O_4 , and $\gamma-Fe_2O_3$ NPs can induce the repression of oxidative stress and the antioxidant system in various crops [78]. Cai et al. [61] studied the foliar application of Fe_3O_4 NPs treatment on tobacco leaves; the results show that this treatment induced

the production of ROS, which could be related to the induction of resistance in tobacco. ZnO and SiO₂ NPs accumulated hydrogen peroxide, even without the presence of the virus. On the other hand, regarding antioxidant enzymes, ZnO, Fe₃O₄, and SiO₂ NPs enhanced the activity of CAT and peroxidase (POD) [9,61]. Other enzymes were expressed in the presence of the virus. SiO₂NP-treated cucumber plants showed *pox* and *pal* gene expression 1 day after PRSV inoculation [74]. Cucumber plants treated with NiONPs after four days of cucumber mosaic virus (CMV) inoculation increased *pod* gene expression [76]. Tomato plants treated with AgNPs and inoculated with ToMV or PVY showed a significant increase in the activity of the antioxidant enzymes POD and polyphenol oxidase (PPO) [62].

6.2. Plant Hormones and Pathogenesis-Related Proteins

Plant resistance operates through an elaborate defense mechanism network, in which salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) regulate the defense pathways to trigger appropriate responses. Other plant growth hormones with the ability to modulate defense responses include gibberellin (GA), auxin (indole-3-acetic acid (IAA)), cytokinin (CK), brassinosteroids (BRs), abscisic acid (ABA), and strigolactone (SL). The crosstalk during multiple plant hormone signals regulates the balance between plant growth and defense. Different types of stress in different hormonal pathways are known to be up or down-regulated hormonal pathways; for example, nanoparticles influence the hormonal balance in plants [80]. *Arabidopsis thaliana* exposed to CuONPs elevates the content of SA and JA, as well as the transcription of genes involved in their signaling routes [81]. Furthermore, the mixture of chitosan polyvinyl alcohol hydrogels (Cs-PVA) and copper nanoparticles (CuNPs) overexpresses the JA gene in tomato plants under salt stress [82]. Fe₃O₄NPs elevates the ABA and SA levels in *Nicotiana benthamiana*, while there was no change in the level of JA [61]. The above discussion suggests that the expression of a particular hormone depends on the specific interaction between the plant and MeNPs, including the dose and the time of application.

Plants treated with AgNPs also increase other hormones, such as CK in *Capsicum annuum* L [83]. IAA and ABA decreased in cotton plants after CeO₂NPs exposition [84]. Small doses of ZnONPs produce an elevation in CK biosynthesis, and at moderate and high doses, ZnONPs function as stressors that stimulate plant defenses, up-regulating the levels of the stress hormones ABA and SA [81]. Plants use mechanisms to modulate plant growth and to regulate the plant's defense against pathogens, including viruses. TMV-infected tobacco with Fe₂O₃ and TiO₂ NPs impacts the phytohormone levels of zeatin riboside, abscisic acid, and brassinoid [72]. TuMV-infested *N. benthamiana* leaves treated with Fe₂O₃ and TiO₂ NPs increased the concentration of phytohormones zeatin ribose (ZR) and BR, and decreased the ABA concentration. Furthermore, infected tomato plants treated with AgNPs increase the proline content [62].

Pathogenesis-related proteins are components of a plant's innate immune system and are part of the diagnostic molecular markers of defense signaling pathways. The expression of the genes *PR1*, *PR2*, and *PR5* indicates the activation of an SA signaling pathway [85]. Uninfected *Nicotiana benthamiana* plants treated with ZnO and SiO₂ NPs up-regulate SA-inducible pathogenesis-related genes *PR1* and *PR2* [9], where the same effect is reported due to the impact of Fe₃O₄NPs [61]. *Cucumis sativus* treated with SiO₂NPs and also inoculated with PRSV (1 dpi) induce the expression of *PR1* [74]. Cucumber plants also increase *PR1* after 2 and 4 dpi with a CMV infection [76].

6.3. Secondary Metabolite Production

There have been a few studies related to the impact of MeNPs on the secondary metabolism of plants, but the mechanism is still not well understood. Some authors indicate that ROS production due to the interaction with NPs will likely interfere with the secondary plant metabolism [86]. Phenylalanine ammonia-lyase (PAL) is an essential enzyme in a plant's defense as a branch point between primary and secondary metabolism. PAL catalyzes the non-oxidative deamination of phenylalanine to *trans*-cinnamate. In the phenylpropanoid pathway, it is also involved in the biosynthesis of SA. It is a SA/JA-inducible enzyme and is also produced by abiotic and biotic stress. Treatments with

SiO₂NPs increase JA-inducible PAL in plants infected with PRSV [74] and in cucumber plants infected with CMV and treated with NiONPs [76]. CuONPs upregulate the *pal* gene and enhance phytochemicals (i.e., glucosinolates and phenolic compounds) in *Brassica rapa* [87]. Tobacco plants show PAL enzyme activity, as well as phenolic compound and flavonoid accumulation, when ZnONPs are applied [88]. Furthermore, CuNPs have a beneficial effect on the accumulation of bioactive compounds in *Moringa oleifera* leaves [89]. ZnONPs also increase the total phenolic and anthocyanin content in potato plants [90].

7. Effect of MeNPs as Biostimulants in Virus-Infected Plants

According to du Jardin [91], plant biostimulants are substances and materials, other than nutrients and pesticides, that when applied to a plant, seeds, or growing substrates in specific formulations, can modify the physiological processes of plants in a way that provides potential benefits to growth, development, and defense responses. Depending on the nanoparticle and the state of the plant, NPs provide a positive (eustress) or negative (distress) impact. [80,92]. Studies on healthy tobacco plants treated with SiO₂, ZnO, and Fe₂O₃ NPs increased tobacco growth [9,61]. Therefore, this biostimulant effect can cause eustress in healthy and also in stressed plants, for example plants infected with a virus, since it counteracts the destructive effects they cause. Infected cucumber plants, which were applied to NiONPs by foliar spray and soil drench showed higher fresh and dry weights and an increase in the number of leaves [76]. TuMV-infested tobacco that was foliar-spray-treated with 50 mg/L of TiO₂ and FeO₃ NPs increased in fresh and dry weight, while the treatment of 200 mg/L had no effect compared to the plants without a nanoparticle treatment [72]. The application of AgNPs to PVY-infected tubers improves the quality parameters compared to infected plants without treatment. The above may be due to the induction of resistance or the impact of NPs on virus entry [67].

8. Nanoparticles against Plant Viral Pathogens

There are several applications of nanotechnology in agriculture, for example (1) in nutrition having beneficial effects due to the production of primary and secondary metabolites; (2) defense against pathogens, as antimicrobial, antifungal, and antiviral agents or as protectants and nanocarriers for insecticides, fungicides, herbicides, and RNA-interference molecules; and (3) the management of the maturation process of fruits in pre- and post-harvest stages [93].

The current use of insecticides is very limited, even in developed countries, due to the problems they cause and because a decrease in a vector population does not necessarily achieve a proportional reduction in the incidence of the virus. Other issues are: (a) the treatment cost, (b) the potential damage to natural enemies of vectors, (c) risk to the environment, and (d) the potential damage to human health [94]. The use of nanoparticles has many advantages due to its low cost, high stability, incorporation of both hydrophilic and hydrophobic substances, secure handling, and they are renewable; the physicochemical properties can also be controlled. The current rate of development of antiviral agents is promising due to the constant appearance of emerging or re-emerging viruses [48]. Due to the above, the use of metal nanoparticles is an exciting opportunity for novel antiviral therapy uses in crops; however, it is essential to take into account its toxicity and manage the application doses. Many of the MeNPs and MoNPs do not present toxicity to humans at the concentrations used as antimicrobials, and many of these concentrations are also those used to activate the antiviral activity, which is an advantage for their large-scale use [95]. Furthermore, the concentrations at which they show antiviral activity do not have negative impacts on plants and sometimes even show a positive or eustress effect.

However, despite the advantages that potentially harmful aspects of the MeNPs present must be considered. Fate should be monitored as well as their potential toxicity and levels of overdosing. Plants can absorb and translocate MeNPs to different parts depending on their bio-ability, concentration, solubility, and exposure time [96]. It is essential to take the doses and the time of application into account and monitor the phenological status of the plant to determine whether they are displaying

any negative effects. It is also necessary to establish the process of accumulation of the nanoparticles, especially in the edible parts of the plant, and even the effect of MeNPs at the nutraceutical level, such that they are not toxic and may even have a beneficial impact for consumers. Although there are no reports related to the time in which MeNPs remain in the plants, nanoparticles can accumulate in fruits and seeds. Studies have reported the transgenerational effects of CeO₂NPs in second-generation seedlings. Those were grown with seeds from parental plants treated with 10 mg/L CeO₂NPs showing more cerium compared to non-treated plants [97]. MeNPs are taken up and accumulate in plants that are food for insects, animals, or humans entering the food chain, and some of them can damage some biological entities of the food chain. Studies have reported that crickets fed NP CeO₂-exposed zucchini leaves accumulate more Ce than bulk exposed insects. The authors indicate that the accumulation of CeO₂NPs in zucchini results in trophic transfer and can contaminate the food chain [98].

The nanoparticles, once used, are released into the environment. Their use and release into the soil have an adverse effect on soil microbial ecology, modifying their number, biomass, activity, and diversity. MeNPs such as ZnO, TiO₂, CeO₂, and Fe₃O₄ NPs affect soil enzyme activities and change soil bacterial communities [99]. Besides, CeO₂NPs have been found to compromise soil fertility [100]. Because of this, it is a critical point for FDA approval of the detailed knowledge of the biodegradation rate [101]. Some polymer encapsulated metal oxide nanoparticles have a degradation time in different media variables from a few days to months [101]. Knowing the biodegradation time of nanoparticles and the damage that may be caused to the potentially susceptible biota, the generation of the application management program to reduce the potential toxicity in the biological communities involved should be an important commitment.

It has been reported that nanoparticle formulations are 10–100 times more effective than traditional formulations to contend with similar problems. Some of these formulations can be used at the rate of 50 mL of nanoemulsions or 8 g per hectare [102]. Studies have reported antiviral activity of MeNPs at a maximum concentration of 200 µg/mL; according to these data, it was established that the amount that could be used for virus control in agriculture is approx. 20–40 g/ha for each application. The large-scale application of nanoparticles in agriculture requires mass production. The low-cost mass production of nanoparticles has gained much attention in industrial and academic circles [103]. Physical methods for the synthesis of nanoparticles are expensive, require strong material, toughness, and strength, and are difficult to facilitate on a large scale, while chemical methods are used in mass production; they could have a negative impact on the environment [104]. An alternative to the use of nanoparticles that harm the environment is an environmentally friendly green approach for their synthesis [105]. The production of MeNPs through biogenesis has advanced in aspects of profitability, and eco-friendly since bacteria, fungi, and plants are involved. However, there are other aspects to improve, green synthesis using plant extracts can be comparable to conventional methods, but mass production requires highly reactive substances or energy-consuming [106].

The ability to act by interfering with any of the stages of the infection to prevent the induction of virus symptoms, to be selective to avoid damaging the host, to have a broad spectrum, and have no harmful effects on the environment would be the requirements to finally achieve a curative antiviral chemical. One of the most promising strategies for the effective use of antivirals is to interfere with virus recognition and entry into the host [48]. Metallic and metal oxide nanoparticles appear to interfere with mammalian virus recognition and have the ability to interact with surface proteins through their interactions with viral envelope glycoproteins and glycoprotein receptors. In addition to their use in crops, MeNPs can be used to prevent viruses in seeds. In plants, vertical transmission through seeds is common. The mechanisms of transmission are through contamination of the seed coat or invasion of the seed embryo indirectly by infection of plant gametes before fertilization or directly from the mother plant to the embryonic tissue after fertilization [107]. The potential use of MeNPs as seed antivirals due to their antimicrobial properties and their size may limit the pathogen in the seed. MeNPs can also be applied in seedlings to avoid virus contamination [65]. In vitro culture represents the most successful approaches for virus eradication in seedling [27]. The use of nanoparticles as compounds to control

the virus can be an alternative to antiviral compounds. On the other hand, studies have reported that the antiviral activity of MeNPs is higher when applied in cultures before infection [9,61]. Therefore, the use of MeNPs is proposed as a preventive control that can be applied to early-stage plants, either to seeds or seedlings. These types of particles presents a wide range of infectious diseases. Preventive control will not only avoid infection by viral pathogens but also by fungi or bacteria [108].

9. Conclusions

The use of NPs has excellent potential to deal with the problem of virus-caused diseases in agriculture. It is necessary to establish precise complementary methodologies such that the technology is ready to be used without risks to the environment or consumers. Among this complementary knowledge, it is required to prescribe doses, the stage of phenological development of the plant to make the applications, and the precise types of NPs that produce the greatest advantages.

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