

Review Article

MANAGEMENT OF PLANT DISEASES WITH GREEN SYNTHESIZED NANOPARTICLES USING PLANT EXTRACTS

Abstract

Nanoparticles (NPs) can typically be synthesized using two different techniques. The first method, the top-down approach, involves physical processes like sonication, laser ablation, radiation, and thermal decomposition. The distinctive features of nanoparticles, such as large surface area to volume ratio, surface plasmon resonance (SPR), and their unique biological, optical, and electrical properties, have significantly increased their demand. Furthermore, the production of nanoparticles has garnered substantial popularity among scientists and researchers worldwide in recent years. The green synthesis of nanoparticles is often referred to as biosynthesis, involving a range of biological sources. In green nanoparticle synthesis, microorganisms or their derivatives, as well as plant extracts, are used instead of synthetic chemicals, with minimal impact on human health and the environment. There is a lack of comprehensive knowledge regarding the extended or prolonged effects of GSNPs on plants and the environment. This knowledge gap hinders our understanding of how GSNPs may impact crops and ecosystems over extended periods. Research efforts should address these questions to ensure the safe and effective utilization of GSNPs in agriculture and environmental applications.

Comment [mj1]: No information about bottom-up approach. Add some information

Comment [mj2]: Write full form once

Keywords: Nanoparticles, sonication, synthetic chemicals, environmental applications

INTRODUCTION

Agriculture faces numerous hurdles, including inefficient use of resources, expensive capital equipment, threats from pests and environmental factors, and a declining interest among the younger generation. In this context, the adoption of cutting-edge technologies plays a crucial role in overcoming these agricultural challenges and boosting crop yields. Nanotechnology is a rapidly advancing field that has effectively tackled various issues across multiple industries, including agriculture (Begum and Jayawardana, 2023). Nanotechnology involves working with nanomaterials, the particles typically having sizes ranging from 1 to 100 nanometers (Hossain *et al.*, 2019). Prof. Norio Taniguchi coined the term nanotechnology, and Richard Feynman is known as the father of nanotechnology. The distinctive features of nanoparticles, such as large surface area to volume ratio, surface plasmon resonance (SPR), and their unique biological, optical, and electrical properties, have significantly increased their demand. Furthermore, the production of nanoparticles has garnered substantial popularity among scientists and researchers worldwide in recent years (Jadoun *et al.*, 2021). Nanotechnology has found widespread applications

in everyday life and is progressively becoming more significant in society. It is being utilized in various fields, including medicine, pharmaceuticals, electronics, energy, environmental sciences, chemistry, food industries, and, more recently, agriculture (Hernandez Diaz *et al.*, 2021). In agriculture, nanotechnology plays a role in the production of nanoscale fertilizers, pesticides, and herbicides (Elemikee *et al.*, 2019). Nanoparticles are used in plant disease management as well.

SYNTHESIS OF NANOPARTICLES

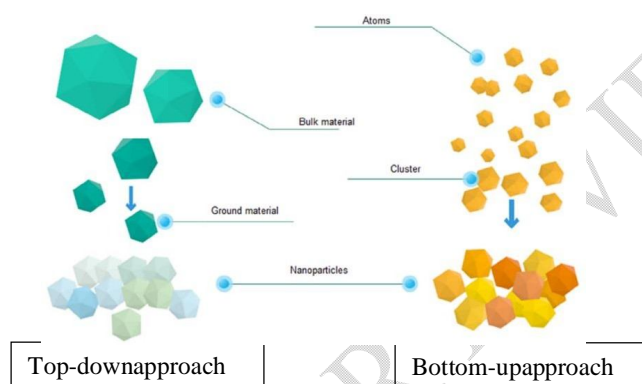


Fig.1-Techniques of nanoparticle synthesis

Nanoparticles (NPs) can typically be synthesized using two different techniques. The first method, the top-down approach, involves physical processes like sonication, laser ablation, radiation, and thermal decomposition. In this approach, NPs are generated by reducing the size of a larger material, resulting in agglomerates with nano-sized particles. Drawbacks of this method are, producing a variety of particle sizes (polydispersity), introducing imperfections (including contamination from the initial material), requiring significant energy and specialized laboratory equipment, and being costly (Pirtarighat *et al.*, 2019). The second approach, the bottom-up method, involves building nano-scale structures from atomic and molecular components. NPs are formed through chemical and biological synthesis. Chemical synthesis techniques include electrochemistry, vapor flux condensation, the sol-gel method, and chemical reduction. Among these, chemical reduction, which utilizes chemicals like sodium borohydride and sodium citrate (Wuithschick *et al.*, 2015), is one of the most commonly used methods for NP generation. Nonetheless, chemical methods often involve multiple chemical species or molecules, which can increase particle reactivity and toxicity. They may also ~~have adverse effects on~~ adversely affect human health (Fu *et al.*, 2014) and the environment due to the decomposition of chemical groups, the generation of by-products, and high energy demand (Rahimi and Doostmohammadi, 2020).

Different methods of nanoparticle synthesis

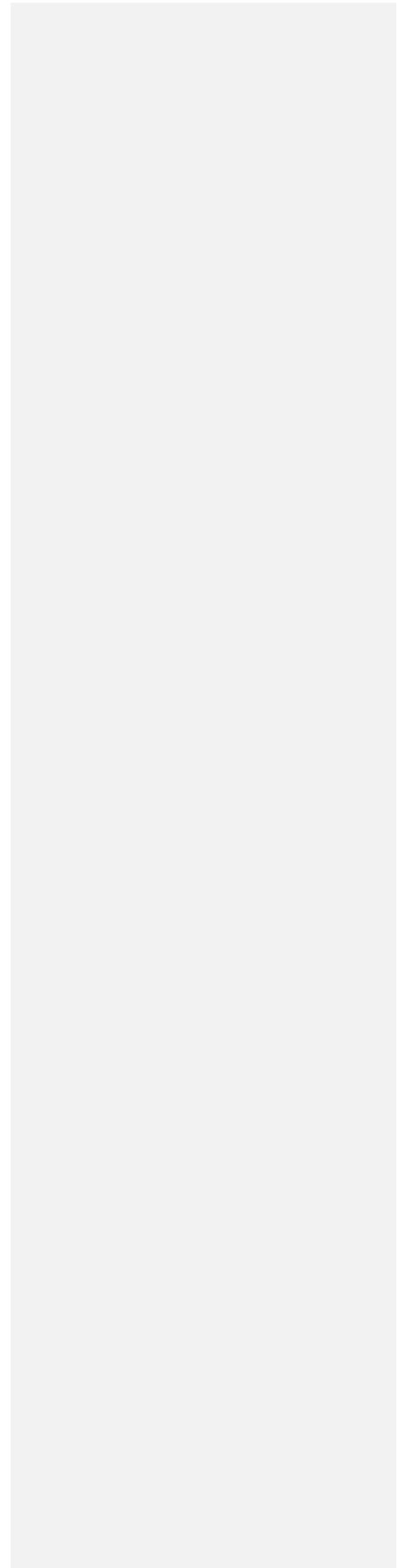
Nanoparticles can be synthesized using various methods, generally categorized into physical, chemical, and biological methods. These methods employ different sources ~~as reducing agents or electron donors for the synthesis process~~ for the synthesis process, such as reducing agents or electron donors. Some of the methods are listed below.

Methods of nanoparticle synthesis (Table 1)

Methods of nanoparticle synthesis		
Physical methods	Chemical methods	Biological methods
<ul style="list-style-type: none"> • Gas phase deposition • Electron beam lithography • Powder ball milling • Pulsed laser ablation • Aerosol 	<ul style="list-style-type: none"> • Coprecipitation • Sonochemical • Thermal decomposition • Microemulsion • Hydrothermal • Electrochemical deposition 	<ul style="list-style-type: none"> • Plants • Fungi • Algae • Bacteria • Biopolymers

(Jadounetal.,2021)

UNDER PEER REVIEW



Even though all these methods have been used for nanoparticle synthesis, the widespread application of physical and chemical approaches in agriculture and healthcare has been relatively limited (Li *et al.*, 1999). The challenges linked to the synthesis of nanoparticles by physical and chemical methods stem from several factors. These include the use of potentially hazardous chemicals, the need for costly equipment and machinery, the requirement for larger laboratory spaces, demanding processing conditions like high temperature and pressure, and significant energy consumption (Ahmad and Jaffri, 2019; Hossain *et al.*, 2019; Some *et al.*, 2019). Moreover, the time required for synthesis, the generation of harmful by-products, the overall high costs involved, and the adverse environmental effects further compound the difficulties associated with nanoparticle production (Kausar *et al.*, 2022).

GREEN SYNTHESIS OF NANOPARTICLES

The green synthesis of nanoparticles is often referred to as biosynthesis, involving a range of biological sources. In green nanoparticle synthesis, microorganisms or their derivatives, as well as plant extracts, are used instead of synthetic chemicals, with minimal impact on human health and the environment. Utilizing green nanotechnology can be a potent approach to address the intricate scientific and technological hurdles in enhancing the safety of the entire agricultural and food production chain (Choudhary *et al.*, 2021). Dr. Kattesh V. Katti is known as the father of green nanotechnology. Green nanomaterials offer significant potential in developing nano-based pesticide formulations due to their small size, large surface area, and properties that can be tailored for specific targets. This has the potential to enhance the effectiveness, safety, and economic impact of conventional pesticides by prolonging their duration of action, reducing the necessary dosage, enabling the controlled release of active ingredients, ensuring stability, and minimizing runoff and environmental residues (Choudhary *et al.*, 2021).

The green synthesis of nanoparticles involves three key components: reducing agents, solvents, and capping agents. Biomolecules, which can be naturally occurring or produced by plants and microbes, such as polyphenols, flavonoids, terpenoids, tannins, alkaloids, polysaccharides, proteins, amino acids, and vitamins, serve as reducing agents during nanoparticle synthesis (Hossain *et al.*, 2019). These biomolecules reduce metal ions to a zero-valence state, and the functional groups within these primary biopolymers and phytochemicals play a role in stabilizing the resulting nanoparticles. Among various biological methods, utilizing plants for nanoparticle synthesis is preferred due to their widespread availability, safety, lack of toxicity,

and the presence of a wider range of phytoconstituents that can act as reducing agents (Uddin *et al.*, 2021).

The green synthesis of nanoparticles is regarded as a safe approach for nanoparticle production and has numerous advantages compared to physical and chemical methods. This method is environmentally friendly, cost-efficient, provides high yields, steady, involves a simple and easy synthesis process, relies on renewable and biocompatible materials, avoids the use of dangerous toxic chemicals, allows for easy accessibility and easy handling, and is an energy-saving process, among other benefits (Uddin *et al.*, 2021; El-Nagger *et al.*, 2017). However, a few drawbacks are associated with it, such as the potential ecological imbalance caused by the overexploitation of natural biological sources and the seasonal variation in the availability of phytochemicals (Altaf *et al.*, 2021).

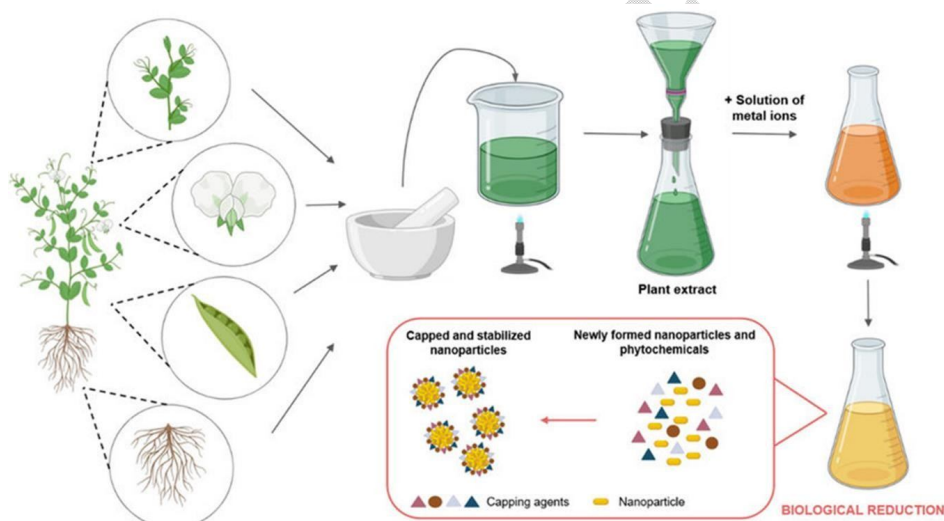
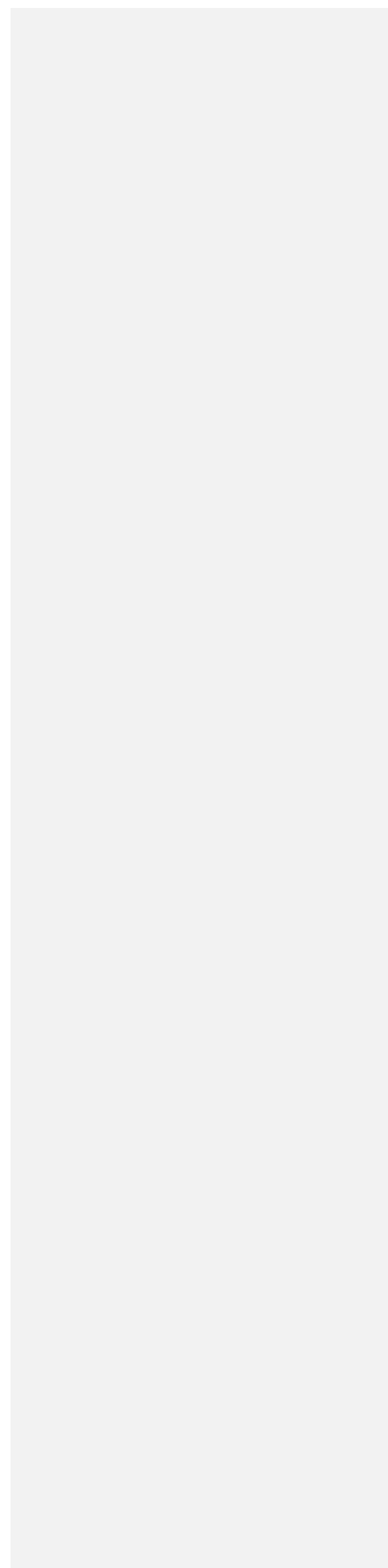


Fig.2-General steps of green synthesis of nanoparticles

Different parts of plants, including fruits, leaves, stems, and roots, have been widely employed for the green synthesis of nanoparticles because of the valuable phytochemicals they contain (Iravani, 2011). In nanoparticle synthesis using plant materials, the specific part of the plant to be used is cleaned and boiled with distilled water. Afterward, the resulting mixture is squeezed, filtered, and combined with the desired solutions for nanoparticle synthesis. As the solution colour changes, indicating the formation of nanoparticles, they can be separated and collected. This natural plant extract-based synthesis is environmentally friendly and cost-

effective, eliminating the need for intermediate chemical reagents. Nanoparticle synthesis occurs in a

UNDER PEER REVIEW



three-step sequence: (i) the reduction of metal ions, often ~~signalled~~ signaled by observable colour changes, (ii) the clustering of nanoparticles; and (iii) the stabilization of these nanoparticles (Some *et al.*, 2019).

FACTORS AFFECTING GREEN SYNTHESIS OF NANOPARTICLES

Solution pH

The pH level of a solution plays a critical role in synthesizing nanoparticles from plant sources (Gerick and Pinches, 2006). It has been emphasized that the pH of the solution can significantly affect the time it takes for synthesis, as well as the size and shape of the resulting nanoparticles (Vijayaraghavan and Ashokkumar, 2017). The formation of nucleation centers during nanoparticle synthesis is highly dependent on the pH level. An increase in pH can lead to the formation of more nucleation centers, which in turn accelerates the reduction of metal ions into metal nanoparticles. The time it takes to reduce metal salts is closely associated with the pH of the reaction medium because pH affects the interaction between the functional groups present in the plant extract and the metal ions (Bali and Harris, 2010). Scientific research has demonstrated that the production of smaller nanoparticles is more likely to occur in a basic solution compared to an acidic one (Dubey *et al.*, 2010).

Reaction time

The incubation and reaction period length plays a crucial role in determining the characteristics, morphology, and yield of nanoparticles (Kuchibatla *et al.*, 2012). Changes in the incubation time and storage conditions also impact the properties of the nanoparticles produced (Mudunkotuwa *et al.*, 2012). Extending the incubation period can result in aggregation and decrease the potential for reducing nanoparticles during the synthesis process (Baer, 2011). Certain research findings have indicated that effective nanoparticle synthesis tends to occur with longer reaction times (Darroud *et al.*, 2011).

Temperature

Temperature is another influential factor in the synthesis of nanoparticles, and it has a similar impact on their morphological properties as pH does. Temperature also affects the formation of nucleation centers, with lower temperatures leading to a reduced formation of these centers, subsequently slowing down the synthesis rate (Pham *et al.*, 2019). Because of the specific secondary metabolites present in plant extracts, it has been recommended to carry out nanoparticle synthesis at room temperature to prevent the degradation and alteration of

functional groups (Jemilugba *et al.*, 2019). Nevertheless, research has demonstrated that triangular-shaped nanoparticles are formed at lower temperatures, whereas spherical-shaped nanoparticles are generated at higher temperatures (Raju *et al.*, 2011). Studies have reported that smaller volumes of plant extract are needed for stable nanoparticle synthesis at high temperatures, and larger-sized nanoparticles tend to be produced at high temperatures (Iravani and Zolfaghari, 2013).

Effect of plant extract concentration

The concentration of plant extract plays a crucial role in synthesizing metal nanoparticles as it provides the electrons needed for reducing metal ions. A reduction in the amount of plant extract results in a reduced formation of nanoparticles (Kiruba *et al.*, 2013). On the other hand, employing a larger volume of plant extract in metal nanoparticle synthesis leads to a higher quantity of phytochemicals, accelerating the reduction of metal salt. Nevertheless, the faster this reduction occurs, the smaller the size of the resulting metal nanoparticles (Din and Rehan, 2017).

Concentration and nature of metal salt

The choice of metal salt employed in the synthesis significantly impacts the characteristics, structure, and size of the nanoparticles produced. For instance, copper salts such as copper chloride, copper sulphate, copper acetate, and copper nitrate are commonly used for nanoparticle synthesis. Research findings have indicated that when copper chloride salt is utilized, triangular and tetrahedral-shaped copper nanoparticles (Cu-NPs) are formed, whereas rod-shaped Cu-NPs are obtained with copper acetate salt (Shankar and Rhim, 2014). When copper sulphate salt is used, spherical Cu-NPs are synthesized (Shah *et al.*, 2015). It has also been reported that an increase in the concentration of metal salt leads to an increase in the size of the nanoparticles (Din *et al.*, 2017).

Pressure

Numerous research investigations have demonstrated that pressure plays a role in influencing the morphological characteristics of nanoparticles synthesized from plant sources (Akintelu *et al.*, 2021).

CHARACTERIZATION OF NANOPARTICLES

Metal nanoparticles are characterized for several purposes, including tracking the completeness of reduction, identifying the functional groups involved in the bio-reduction process, assessing purity levels, and analyzing their morphological attributes. The commonly employed techniques for these purposes are highlighted below;

UV-visible spectrophotometry

The interaction between plant extract and metal salt results in observable colour changes in the solution due to the excitation of surface plasmon vibrations in the NPs. The absorption band corresponding to each metal can be confirmed by analyzing the solution with a UV spectrophotometer. UV-visible spectrophotometry tracks the characteristic peaks generated by metal salt-derived nanoparticles (NPs) at various absorption wavelengths during the synthesis process. For instance, in the case of Cu-NPs, a distinctive absorption occurs in the range of 520-600 nm within the visible region due to surface plasmon resonance (SPR). UV-visible spectroscopy is also employed to estimate the aggregation state, size, and size distribution of NPs (Lotha *et al.*, 2019).

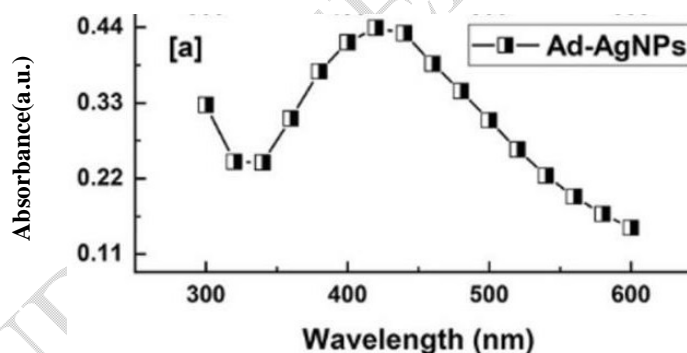


Fig.3-UV visible absorption spectra of silver nanoparticles green synthesized from *Amaranthus dubius*

Comment [mj3]: Add author name

Fourier transform infrared (FTIR) spectroscopy

FTIR spectroscopy can be used to detect functional groups capable of donating electrons for the reduction of metal salts (Sebeia *et al.*, 2019). FTIR spectrophotometer measures the wavelength of light against infrared intensity. Researchers typically compare the FTIR spectra

of the plant extract and the synthesized nanoparticles to determine which functional groups are responsible for reducing the metal ions (Akintelu *et al.*, 2020).

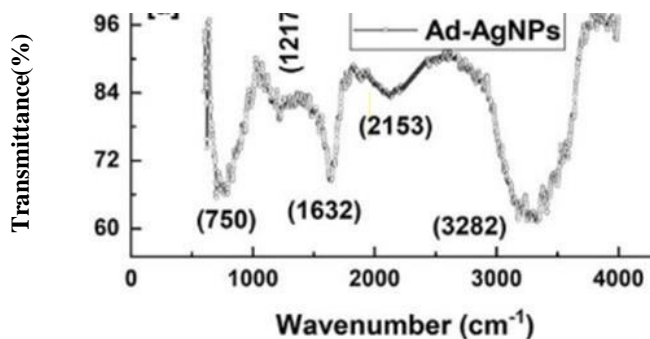


Fig.4-FTIR spectra of silver nanoparticles green synthesized from *Amaranthus dubius*

X-ray diffraction (XRD)

The X-ray diffraction (XRD) technique is utilized to gather structural data regarding the crystalline nature of nanoparticles (Sharma *et al.*, 2019). During XRD analysis, the high-energy X-ray rays emitted by the machine penetrate deeply into the nanoparticles, yielding valuable insights into their structure (Jamdade *et al.*, 2019). The formation of nanoparticles in the nanoscale range is typically characterized by broadening the peaks observed in XRD analysis.

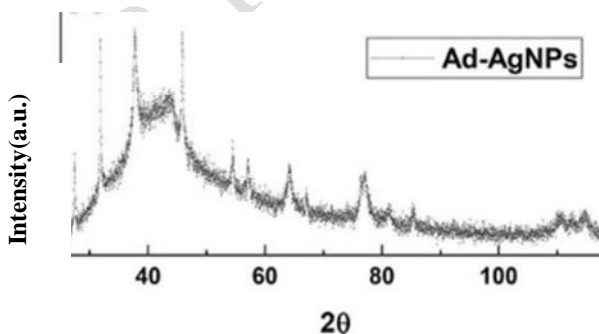


Fig.5-X-ray diffraction analytical pattern of silver nanoparticles green synthesized from *Amaranthus A. dubius*

Scanning electron microscopy (SEM)

The morphological characteristics of nanoparticles are assessed through the use of scanning electron microscopy (SEM). Additionally, SEM analysis can be employed to estimate the average size of nanoparticles with the assistance of certain statistical software tools (Akintelu *et al.*, 2021).

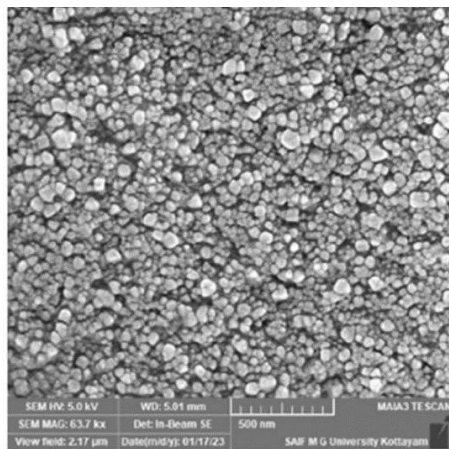


Fig.6- FESEM image of silver nanoparticles synthesized from *Amaranthus dubius*

Transmission electron microscopy (TEM)

TEM provides higher magnification, superior resolution, and more precise information regarding shape, crystallinity, and size compared to SEM (Akintelu *et al.*, 2021). Additionally, TEM is particularly advantageous due to its ability to distinguish between crystalline and amorphous structures using selected area electron diffraction techniques, which makes it even more beneficial for nanoparticle characterization (Caroling *et al.*, 2015).

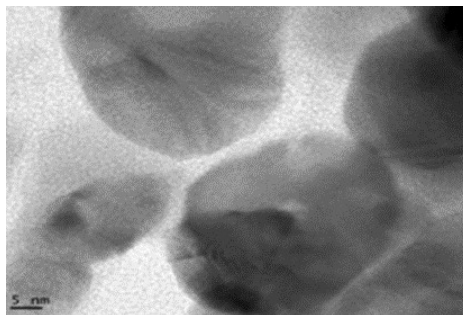


Fig.7- TEM image of silver nanoparticles synthesized from *Amaranthus dubius*

Atomic force microscopy (AFM)

Atomic force microscopy (AFM) is used for morphological assessment of nanoparticles (Chidanandappa and Nargund, 2020).

Energy dispersive X-ray spectroscopy (EDX)

EDX spectroscopy is widely accepted as a suitable method for determining the elemental composition of nanoparticles. This is achieved by examining the distinct groups of peaks in the X-ray spectrum produced by the unique atomic structure of each element, facilitating the identification of these elements (Noruzi, 2015).

MECHANISM OF ACTION OF NANOPARTICLES AGAINST PHYTOPATHOGENS

Green-synthesized nanoparticles (GSNPs) have primarily been studied for their effectiveness in combatting phytopathogens. However, our understanding of how these nanoparticles inhibit or kill microorganisms remains limited. The mechanism of action of nanoparticles can be broadly categorized as disruption of the peptidoglycan layer in bacterial cell walls, toxicity resulting from the release of toxic metal ions into the cytoplasm leading to imbalances in nutrient uptake, impairment of membrane function including membrane damage and the loss of membrane potential, generation of reactive oxygen species (ROS) and the production of antioxidants, damage to genetic material such as double-helix strand breaks, dysfunction of proteins, etc.

Apart from the impact of metal ions, different metabolites found in plant extracts have been observed to induce cell death in pathogens and stimulate systemic resistance in plants (Mishra *et al.*, 2017). Alkaloids, phenolics, and natural compounds present in plant extracts have been shown to possess bactericidal and fungicidal properties against plant pathogens, thereby enhancing the efficacy of green-synthesized nanoparticles (Choudhary *et al.*, 2021).

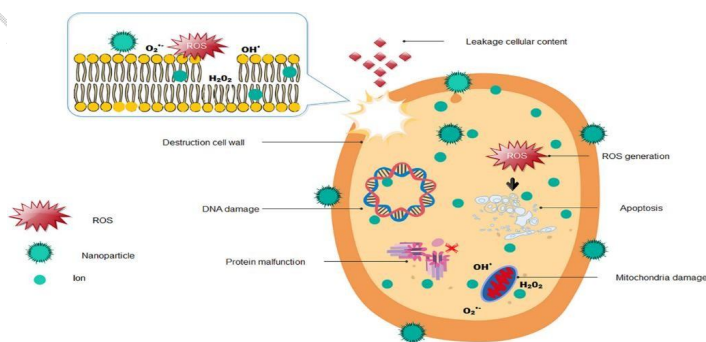


Fig.8-Mechanism of action of nanoparticles against phytopathogens

MECHANISM

OF ACTION OF DIFFERENT METAL NANOPARTICLES AGAINST PHYTOPATHOGENS (Table 2)

Nanoparticle type	Ionic form	Mechanism of action	Phytopathogen type
AgNPs	Ag ⁺	<ul style="list-style-type: none">• Release of ions that are toxic towards pathogens• Increase the permeability of the bacterial membrane• Disruption of the bacterial membrane• Damage to the cell components (lipids, DNA and proteins)• Inhibition of enzyme activity• Inhibition of DNA replication	Bacteria and fungi
Al ₂ O ₃ NPs	Al ³⁺	<ul style="list-style-type: none">• Release of ions that are toxic towards pathogens• Increase ROS production• Depolarization of cell membranes	Bacteria
AuNPs	Au ³⁺	<ul style="list-style-type: none">• Disruption of bacterial membrane and alteration of metabolism• Damage to cell organelles (cell wall and mitochondria)• Inhibition of DNA uncoiling and transcription mediated by binding of AuNPs to bacterial DNA	Bacteria
CeO ₂ NPs	Ce ³⁺ , Ce ⁴⁺	<ul style="list-style-type: none">• Inhibition of ion transport through pumps• Induction of oxidative degradation of lipids and/or proteins of the pathogen's plasma membrane• Impairment of electron flux and bacterial respiration• Inhibition of fungal enzyme activity	Gram-positive bacteria and fungi

CdONPs	Cd ²⁺	<ul style="list-style-type: none"> • Release of ions that are toxic against pathogens • Induction of oxidative stress on bacterial cells • Increase ROS production • Interrupted transmembrane electron transport and mitochondria damage 	Bacteria
CuNPs/CuONPs	Cu ²⁺	<ul style="list-style-type: none"> • Inhibition of enzyme activity essential to hemicroorganisms • Increase ROS production • Damage to essential molecules such as DNA 	Bacteria and fungi
SeNPs	Se ⁶⁺ , Se ⁴⁺	<ul style="list-style-type: none"> • Intracellular ATP depletion • Induction of oxidative stress through ROS production • Alteration of bacterial membrane potential • Disruption of bacterial membrane • Inhibition of fungal spore germination 	Bacteria and fungi
SiNPs/SiO ₂ NPs	Si ⁴⁺	<ul style="list-style-type: none"> • Induction of mechanical damage to bacterial membrane • Increased ROS production • Induction of oxidative stress 	Bacteria and fungi
TiO ₂ NPs	Ti ⁴⁺	<ul style="list-style-type: none"> • Increase in ROS production • Induction of photocatalytic damage 	Bacteria and fungi
ZnONPs	Zn ²⁺	<ul style="list-style-type: none"> • Release of ions that are toxic towards pathogens • Increased ROS production • Disruption of mitochondrial function • Induction of changes in cell morphology and release of cell components 	Bacteria

Fe ₂ O ₃ NPs	Fe ³⁺ , Fe ²⁺	<ul style="list-style-type: none"> • Induction of oxidative stress through ROS production • Destruction of cell membranes, inducing changes in cell morphology and release of cell components • Damage to essential molecules such as proteins and DNA • Release of iron ions leading to oxidative damage by Fenton reaction 	Bacteria and fungi
------------------------------------	--	--	--------------------

(Hernandez Diaz *et al.*, 2021)

ANTIMICROBIAL AND DISEASE-MANAGING PROPERTIES OF NANOPARTICLES GREEN SYNTHESIZED FROM PLANT EXTRACTS

Green synthesized nanoparticles are reported to be effective against several plant pathogenic fungi, bacteria, and viruses.

GREEN SYNTHESIZED NANOPARTICLES AGAINST PHYTOPATHOGENIC FUNGI (Table 3)

Plant	Plant part used	NP	Pathogen	Effective concn.	Experimental approach	Reference
<i>Oryza sativa</i>	Leaf	Ag	<i>Rhizoctonia solani</i>	-	-	Kora <i>et al.</i> , 2020
<i>Solanum tuberosum</i>	Leaf	Ag	<i>Alternaria alternata</i> , <i>R. solani</i> , <i>Botrytis cinerea</i> , <i>Fusarium oxysporum</i>	22.8 µg/mL	<i>In vitro</i>	Almadiy and Nenaah, 2018
<i>Abelmoschus esculentus</i>	Seed	Au	<i>Puccinia graminis tritici</i> , <i>Aspergillus flavus</i> , <i>Aspergillus niger</i> , <i>Candida albicans</i>	-	-	Jayaseelan <i>et al.</i> , 2013

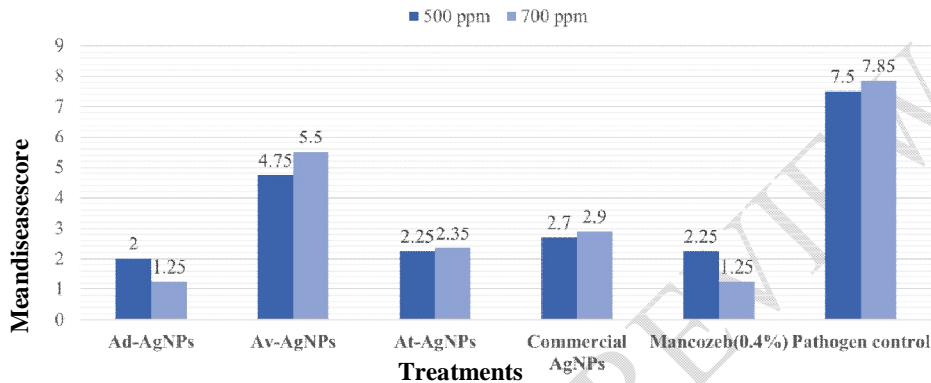
<i>Citrusi nensis</i>	Fruit	Cu	<i>Colletotrichum capsici</i>	-	<i>Invitro</i>	Divteet al.,2019
<i>Aloevera</i>	Leaf	Se	<i>Colletotrichum coccodes, Penicillium digitatum</i>	-	<i>Invitro</i>	Fardsadegh andJafarizadeh - Malmiri, 2019
<i>Curcuma longa</i>	Roots	TiO ₂	<i>Fusariumgraminearum</i>	0.2–20 mg/mL	<i>Invitro</i>	Abdul et al.,2016
<i>Ecliptaalba</i>	Leaf	ZnO	<i>Sclerospora graminicola</i>	-	Field	Nandhini et al., 2019
<i>Punicagr anatum</i>	Peels	ZnO	<i>Aspergillus niger</i>	50 µg/mL	<i>Invitro</i>	Mishraand Sharma,2015

Silver nanoparticles green synthesized with leaf extract of disease-resistant amaranthus genotypes effectively suppress leaf blight (*Rhizoctonia solani*) disease in a susceptible red amaranthus cultivar (Divya et al., 2023)

Silver nanoparticles (AgNPs) were produced through green synthesis using leaf extracts from different types of amaranthus plants: the leaf blight disease-susceptible red amaranthus (*Amaranthus tricolor* L.), the disease-resistant green amaranthus (*A. dubius*), and the wild amaranthus (*A. viridis*) genotypes. These nanoparticles were then characterized using various techniques and evaluated for their antifungal effects against the pathogen *Rhizoctonia solani*.

The green-synthesized nanostructures exhibited absorption characteristics typical of silver nanoparticles in spectroscopy and demonstrated a face-centered cubic structure in X-ray diffraction analysis. Field Emission Scanning Electron Microscopy and High-Resolution Transmission Electron Microscopy revealed that the nanoparticles had a size range of 35–45 nm across all samples.

In vitro experiments using a poisoned-food assay showed that the nanoparticles inhibited the mycelial growth of the pathogen when applied at concentrations of 500 and 750 ppm. Moreover, detached leaves from the red amaranthus variety were sprayed with the nanoparticles and then exposed to the pathogen. It was observed that leaves treated with Ad-AgNPs and Av-AgNPs had significantly fewer lesions compared to those treated with At-AgNPs.



Graph 1: Leaf blight incidence in *Amaranthus-tricolor* var. Arun in the detached leaf assay

Mycelial growth inhibition of the amaranthus leaf blight pathogen *Rhizoctonia solani* in poisoned-food assay (Table 4)

Treatments	Percentage disease index			
	500 ppm		750 ppm	
	3DAI	5DAI	3DAI	5DAI
Ad-AgNPs	1.64±1.42 ^d	16.04±1.23 ^e	1.64±0.71 ^c	7.40±2.13 ^e
Av-AgNPs	4.11±0.71 ^d	24.28±1.88 ^d	2.46±0.00 ^c	17.69±1.88 ^d
At-AgNPs	16.45±3.10 ^b	51.44±1.42 ^b	8.77±0.23 ^b	49.38±2.13 ^b
Commercial AgNPs	10.29±0.72 ^c	30.04±1.42 ^c	8.23±0.71 ^b	27.57±1.88 ^c
Mancozeb(0.4%)	4.93±2.47 ^d	10.69±1.42 ^f	1.23±1.23 ^c	6.99±2.56 ^e
Pathogen Control	39.91± 377 ^a	84.36±5.70 ^a	11.93±1.42 ^a	86.83±1.42 ^a
Absolute Control	0.00	0.00	0.00	0.00

In an *in vivo* assay, where susceptible red amaranthus plants were challenged with the pathogen after foliage spraying with AgNPs at a concentration of 750 ppm, the lowest disease index (7.40) was recorded for Ad-AgNPs, followed by Av-AgNPs (17.69) five days after inoculation. In contrast, At-AgNPs-treated plants had a disease index of 49.38.

These findings suggest that the application of AgNPs, green-synthesized using leaf extracts from disease-resistant amaranthus genotypes, effectively reduced the incidence of leaf blight disease in a susceptible amaranthus variety.

GREENSYNTHESIZED NANOPARTICLES AGAINST PHYTOPATHOGENIC BACTERIA
(Table 5)

Plant	Plant part used	NP	Pathogen	Effective concentration-	Experimental approach	Reference
<i>Piper nigrum</i>	Stem	Ag	<i>Citrobacter freundii</i> <i>Erwinia actinida</i>	-	<i>In vitro</i>	Paulkumaret al., 2014
<i>Azadirachta indica</i>	Leaf	Ag	<i>Xanthomonas oryzae</i> pv. <i>oryzae</i>	-	<i>In vitro</i>	Mankadet al., 2020
<i>Phyllanthus emblica</i>	Fruit	Ag	<i>Acidovorax oryzae</i>	30 µg/mL	<i>In vitro</i>	Masumetal., 2019
<i>Cymbopoga citratus</i>	Leaf	Al ₂ O ₃	<i>Pseudomonas aeruginosa</i>	2000 µg/mL	<i>In vitro</i>	Ansarietal., 2015
<i>Gloriosa superba</i>	Leaf	CeO ₂	<i>Pseudomonas aeruginosa</i>	100 µg/mL	<i>In vitro</i>	Arumugumet al., 2015
<i>Carica papaya</i>	Leaf	CuO	<i>Ralstoniasola nacearum</i>	250 µg/mL	<i>In vitro</i> & greenhouse	Chenetal., 2019
<i>Rosmarinus officinalis</i>	Flower	MgO	<i>X.oryzae</i> pv. <i>oryzae</i>	-	<i>In vitro</i>	Abdallahetal., 2019
<i>Withania somnifera</i>	Leaf	Se	<i>Bacillus subtilis</i>	25 µg/mL	<i>In vitro</i>	Alagesana nd Venugopal, 2019
<i>Cynodon dactylon</i>	Leaf	Si	<i>Pseudomonas aeruginosa</i>	60 µg/mL	<i>In vitro</i>	Babuetal., 2018

<i>Trigonella foenum-graecum</i>	Leaf	TiO ₂	<i>Bacillus subtilis</i>	10 mg/mL	<i>Invitro</i>	Subhapriyaan dGomathipriya, 2018
<i>Solanum lycopersicum</i>	Fruit	ZnO	<i>X.oryzaepv. oryzae</i>	-	<i>Invitro</i>	Ogunyemiet al., 2019

Phytofabrication of silver nanoparticles using three flower extracts and their antibacterial activities against pathogen *Ralstonia solanacearum* strain YY06 of bacterial wilt (Cheng et al., 2020)

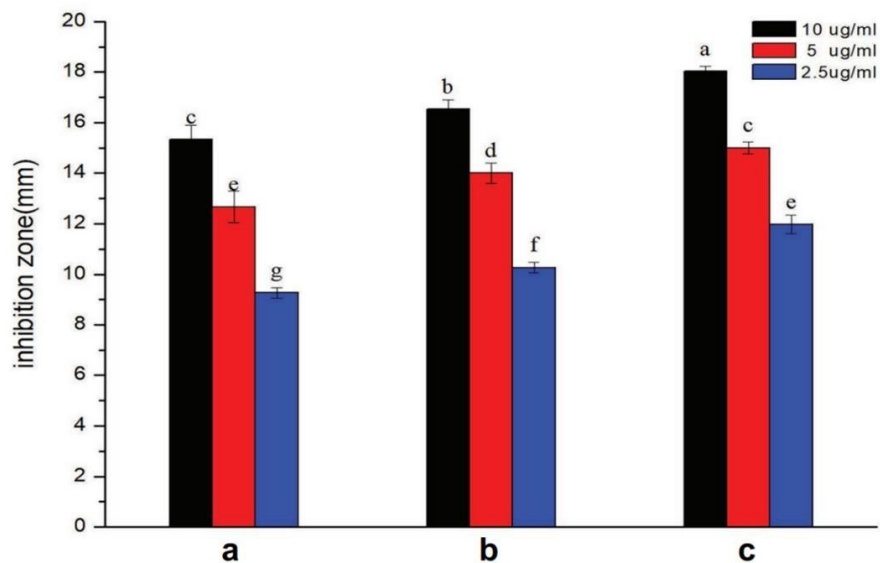
Bacterial wilt, caused by the phytopathogen *Ralstonia solanacearum*, is a significant global problem. The conventional use of bactericides and antibiotics to control bacterial wilt has shown limited effectiveness and posed environmental risks. This study aimed to produce silver nanoparticles (AgNPs) through a natural process using extracts from the flowers of cannalily (*Canna indica* L.), Cosmos (*Cosmos bipinnata* Cav.), and Lantana (*Lantana camara* L.) plants.

The synthesized AgNPs were validated and characterized using various techniques, including UV-visible spectroscopy, Fourier transform infrared spectroscopy (FTIR), X-ray diffraction (XRD), transmission electron microscopy (TEM), and scanning electron microscopy (SEM). UV-visible spectra revealed distinctive absorption peak bands at 448 nm, 440 nm, and 428 nm for AgNPs synthesized using *C. indica* L., *C. bipinnata* Cav., and *L. camara* L. flowers, respectively. FTIR spectra confirmed the involvement of biofunctional groups from the flower extracts in the AgNPs synthesis process, acting as capping and stabilizing agents.

The spherical AgNPs synthesized from the three different flower sources had average diameters of 43.1 nm, 36.1 nm, and 24.5 nm for *C. indica* L., *C. bipinnata* Cav., and *L. camara* L., respectively. The AgNPs produced by *L. camara* L. flowers at a concentration of 10.0 µg/ml exhibited the highest suppression zone of 18 mm against the *R. solanacearum* strain YY06, surpassing the efficacy of AgNPs synthesized by *C. indica* L. and *C. bipinnata* Cav. flowers.

Furthermore, the AgNPs negatively impacted various aspects of *R. solanacearum*, including bacterial growth, biofilm formation, swimming motility, nucleic acid efflux, cell death, cell

membrane damage, and the generation of reactive oxygen species (ROS), mainly when they were present in high concentrations and had small particle sizes.



Graph 2: Diameter of bacterial growth inhibition (mm) caused by AgNPs which were synthesized by (a) *C. indica* L. flower; (b) *C. bipinnata* Cav. flower; and (c) *L. camara* L. flower

In summary, the biosynthesized AgNPs have the potential to serve as an effective and environmentally friendly antibacterial agent for reasonably inhibiting *R. solanacearum*.

GREEN SYNTHESIZED NANOPARTICLES HAVING BOTH ANTIFUNGAL

AND ANTIBACTERIAL PROPERTIES (Table 6)

Plant	Plant part used	NP	Pathogen	Effective concentration-	Experimental approach	Reference
<i>Azadirachta indica</i>	Leaf	Ag	<i>R. solanacearum</i> , <i>Aspergillus</i> sp. <i>Fusarium</i> sp.	-	<i>In vitro</i>	Haroon <i>et al.</i> , 2019
<i>Leucaena leucocephala</i>	Leaf	CdO	<i>P. aeruginosa</i> , <i>Aspergillus niger</i>	500 µg/mL	<i>In vitro</i>	Savale <i>et al.</i> , 2017
<i>Ocimum sanctum</i>	Leaf	Cu	<i>Alternaria carthami</i> , <i>A. niger</i> , <i>Colletotrichum loeosporioides</i> , <i>Colletotrichum lindemuthianum</i> , <i>Drechslera sorghicola</i> , <i>F. oxysporum</i> , <i>Macrophomina phaseolina</i> , <i>Rhizoctonia bataticola</i> , <i>R. solani</i> , <i>Xanthomonas axonopodis</i> sp. <i>citri</i> , <i>X. axonopodis</i> sp. <i>punicae</i>	10–60 µg/mL	<i>In vitro</i>	Shende <i>et al.</i> , 2016

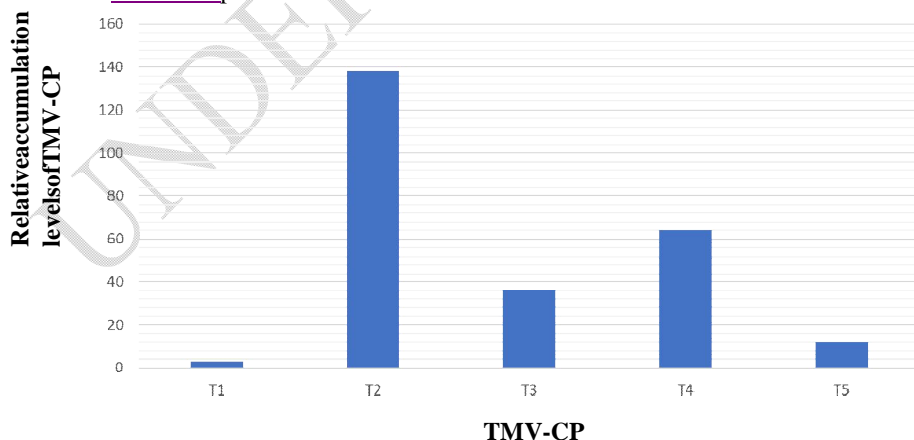
GREEN SYNTHESIZED NANOPARTICLES AGAINST PHYTOPATHOGENIC VIRUSES

Green synthesized ZnO nanoparticles mediated by *Mentha spicata* extract induce plant systemic resistance against Tobacco Mosaic Virus (Abdelkhalek and Al-Askar, 2020)

Globally, plant viral infections pose a significant threat to food security, leading to substantial crop production losses. Nanoparticles have proved to be effective agents for controlling various plant pathogens. However, our understanding of their effects against viral infections is limited. In this study, they achieved the green synthesis of zinc oxide nanoparticles (ZnO NPs) using an aqueous leaf extract of *Mentha spicata*.

X-ray diffraction patterns confirmed that the prepared ZnO NPs had a crystalline structure. Analyses using dynamic light scattering and scanning electron microscopy showed that the resulting ZnO NPs were spherical ~~in shape~~, with particle sizes ranging from 11 to 88 nm. Fourier transform infrared spectroscopy identified different functional groups, capping, and stability agents and revealed the presence of Zn-O bonds at a wavenumber of 487cm^{-1} .

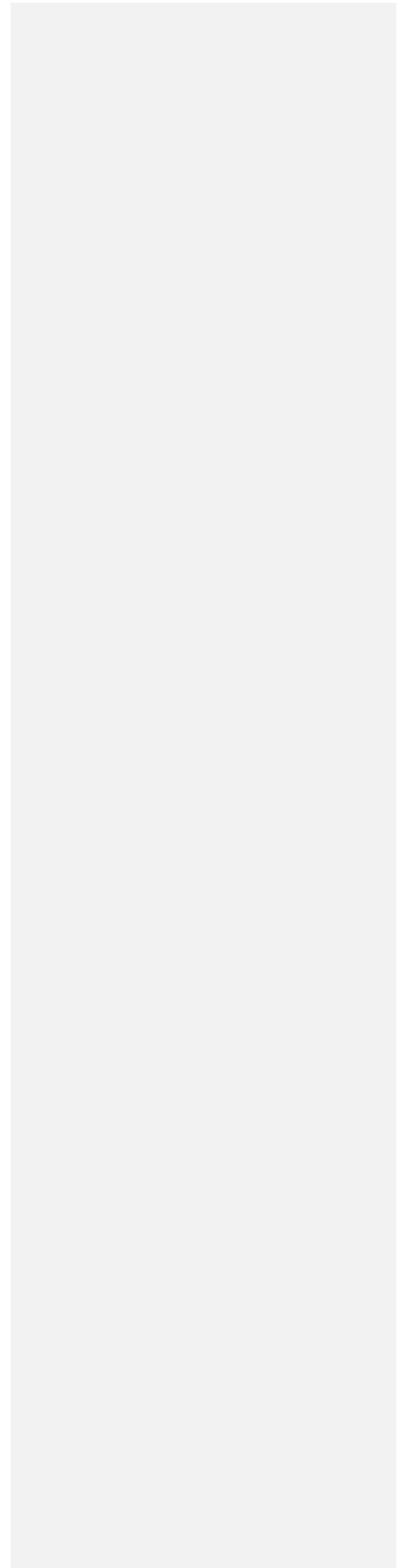
Under greenhouse conditions, we evaluated the antiviral activity of biologically synthesized ZnO NPs (at a concentration of $100\ \mu\text{g/mL}$) against the Tobacco mosaic virus (TMV). The most effective treatment involved double foliar application of the prepared ZnO NPs 24 hours before and 24 hours after TMV inoculation, resulting in a 90.21% reduction in viral accumulation and disease severity. Additionally, the transcriptional levels of genes associated with plant defense mechanisms were induced and up-regulated in all ZnO NPs-treated NP-treated plants.



Graph 3: Relative expression level of TMV-CP gene in TMV-infected tomato plants at 21 days after inoculation (T1 = Mock-treated plants (Control), T2 = plants inoculated with TMV only, T3 = plants treated with ZnO NPs (24 h before TMV inoculation), T4 = plants treated with ZnO NPs

(24 h after TMV inoculation) and T5= plant treated with ZnO NPs (24 h before TMV inoculation and 24 h after TMV inoculation)

UNDER PEER REVIEW



Significantly, results demonstrated that the aqueous extract of *Mentha spicata* served as an effective reducing agent for the green synthesis of ZnO NPs, which exhibited substantial antiviral activity. Ultimately, the protective and curative effects of ZnO NPs against TMV suggest their potential application in managing plant viral diseases.

CHALLENGES

The use of biological sources for nanoparticle synthesis may impose constraints on the possibility of large-scale commercial production of nanoparticles. This limitation arises because the availability and prevalence of the specific plants or biological materials used can fluctuate depending on the season and geographic location. Furthermore, it is crucial to consider the optimal growth stage of these plants when utilizing them for nanoparticle synthesis, as this factor significantly influences the quality and yield of the nanoparticles.

Green-synthesized nanoparticles (GSNPs) can exhibit a wide range of sizes, which can pose challenges in achieving uniformity in their properties for various applications. Additionally, GSNPs are often less stable and prone to oxidation, which can impact their long-term effectiveness and shelf life (Begum and Jayawardana, 2023).

One significant drawback is that many experiments investigating nanoparticles' effects have primarily focused on short-term crop or immediate impacts. This limited scope means that we may not have a comprehensive understanding of the cumulative and long-term effects of nanoparticles on plants, the environment, and even human health. As nanoparticles become more widely used in agriculture and other fields, it becomes increasingly important to conduct research that addresses their potential long-term impacts and cumulative toxicity. This will help to ensure the safe and sustainable application of nanoparticles in various sectors (Choudhary *et al.*, 2021).

FUTURE PROSPECTS

It is essential to conduct field trials with various crops and diseases to evaluate the efficacy of all synthesized nanoparticles compared to commercial pesticides and biocontrol agents. Research should explore the use of less phytotoxic metals like Cu, Zn, Mn, Fe, and Mg as potential alternatives to costly silver nanoparticles (AgNPs). A thorough understanding of the structural properties of green nanoparticles, including morphology, size, functional groups, loading capacity, and their impact on plants, should be established. A more efficient, rapid, and scalable protocol for formulating green nanoparticles is necessary for successful large-scale

production. The effects of nanoparticles on soil, wildlife, plant biodiversity, crop yields, and farmer income should be investigated. The toxicity and effectiveness of green-synthesized nanoparticles should be compared to chemically synthesized nanoparticles to assess their suitability for various applications.

CONCLUSION

Green-synthesized nanoparticles (GSNPs) are known for their ease of production and cost-effectiveness. They offer several advantages, including their ability to induce systemic resistance to diseases and exhibit fungicidal and bactericidal properties. However, despite their promising attributes, questions remain about their efficacy and long-term sustainability when used in field conditions. There is a lack of comprehensive knowledge regarding the extended or prolonged effects of GSNPs on plants and the environment. This knowledge gap hinders our understanding of how GSNPs may impact crops and ecosystems over extended periods. Research efforts should address these questions to ensure the safe and effective utilization of GSNPs in agriculture and environmental applications.

REFERENCE

- Abdallah, Y., Ogunyemi, S.O., Abdelazez, A., Zhang, M., Hong, X., Ibrahim, E., Hossain, A., Fouad, H., Li, B., and Chen, J. 2019. The green synthesis of MgO nano-flowers using *Rosmarinus officinalis* L. (Rosemary) and the antibacterial activities against *Xanthomonas oryzae pv. oryzae*. *BioMed. Res. Int.* 20: 510-536.
- Abdelkhalek, A. and Al-Askar, A.A. 2020. Green synthesized ZnO nanoparticles mediated by *Mentha spicata* extract induce plant systemic resistance against *Tobacco mosaic virus*. *Appl. Sci.* 10(15): 5054-5075.
- Abdul Jalill, R.D., Nuaman, R.S., and Abd, A.N. 2016. Biological synthesis of Titanium Dioxide nanoparticles by *Curcuma longa* plant extract and study its biological properties. *World Sci. News*, 49(2):204-222.
- Ahmad, K.S. and Bibi Jaffri, S. 2019. Carboxen-mediated ZnO nanoparticles: amplified photocatalytic and antimicrobial action. *IET Nanobiotechnol.* 13(2):150-159.

- Akintelu, S.A., Folorunso, A.S., Oyebamiji, A.K., and Olugbeko, S.C. 2021. Mosquito repellent and antibacterial efficiency of facile and low-cost silver nanoparticle synthesized using the leaf extract of *Morinda citrifolia*. *Plasmonics*, 14: 1-12.
- Akintelu, S.A., Oyebamiji, A.K., Olugbeko, S.C., and Latona, D.F. 2021. Green chemistry approach towards the synthesis of copper nanoparticles and its potential applications as therapeutic agents and environmental control. *Curr. Res. Green Sustain. Chem.*, 4: 100176-100189.
- Akintelu, S.A., Olugbeko, S.C., Folorunso, F.A., Oyebamiji, A.K., and Folorunso, A.S. 2020. Characterization and pharmacological efficacy of silver nanoparticles biosynthesized using the bark extract of *Garcinia kola*. *J. Chem.* 20: 1-7.
- Alagesan, V. and Venugopal, S. 2019. Green synthesis of selenium nanoparticle using leaves extract of *Withania somnifera* and its biological applications and photocatalytic activities. *Bionanosci.* 9: 105-116.
- Almadiy, A.A. and Nenaah, G.E. 2018. Ecofriendly synthesis of silver nanoparticles using potato steroidal alkaloids and their activity against phytopathogenic fungi. *Braz. Arch. Biol. Technol.* 61: 112-114.
- Altaf, M., Zeyad, M.T., Hashmi, M.A., Manoharadas, S., Hussain, S.A., Abuhasil, M.S.A., and Almuzaini, M.A.M. 2021. Effective inhibition and eradication of pathogenic biofilms by titanium dioxide nanoparticle synthesized using *Carum copticum* extract. *RSC adv.* 11(31): 19248-19257.
- Ansari, M.A., Khan, H.M., Alzohairy, M.A., Jalal, M., Ali, S.G., Pal, R., and Musarrat, J. 2015. Green synthesis of Al_2O_3 nanoparticles and their bactericidal potential against clinical isolates of multi-drug resistant *Pseudomonas aeruginosa*. *World J. Microbiol. Biotechnol.* 31: 153-164.
- Arumugam, A., Karthikeyan, C., Hameed, A.S.H., Gopinath, K., Gowri, S., and Karthika, V. 2015. Synthesis of cerium oxide nanoparticles using *Gloriosa superba* L. leaf extract and their structural, optical and antibacterial properties. *Mater. Sci. Eng.* 49: 408-415.

- Babu, R.H., Yugandhar, P., and Savithramma, N. 2018. Synthesis, characterization and antimicrobial studies of biosilica nanoparticles prepared from *Cynodon dactylon* L.: a green approach. *Bull. Mater. Sci.* 41:1-8.
- Baer, D.R. 2011. Surface Characterization of Nanoparticles: critical needs and significant challenges. *J. Surf. Anal.* 17(3): 163-169.
- Bali, R. and Harris, A.T. 2010. Biogenic synthesis of Au nanoparticles using vascular plants. *Ind. Eng. Chem. Res.* 49(24): 12762-12772.
- Begum, S.R. and Jayawardana, N.U. 2023. Green synthesized metal nanoparticles as an ecofriendly measure for plant growth stimulation and disease resistance. *Plant Nano Biol.* 3: 10028-10040.
- Caroling, G., Vinodhini, E., Ranjitham, A.M., and Shanthi, P. 2015. Biosynthesis of copper nanoparticles using aqueous *Phyllanthus embilica* (Gooseberry) extract: characterisation and study of antimicrobial effects. *Int. J. Nano. Chem.* 1(2):53-63.
- Chen, J., Mao, S., Xu, Z., and Ding, W. 2019. Various antibacterial mechanisms of biosynthesized copper oxide nanoparticles against soilborne *Ralstonia solanacearum*. *RSC adv.* 9(7): 3788-3799.
- Cheng, H.J., Wang, H., and Zhang, J.Z. 2020. Phytofabrication of silver nanoparticles using three flower extracts and their antibacterial activities against pathogen *Ralstonia solanacearum* strain YY06 of bacterial wilt. *Front. Microbiol.* 11:2110-2123.
- Chidanandappa, V.B. and Nargund, G. 2020. Green synthesis of Chitosan based copper nanoparticles and their bio-efficacy against bacterial blight of pomegranate (*Xanthomonas axonopodis* sp. *punicae*). *Int. J. Curr. Microbiol. App. Sci.* 9(1):1298-1305.
- Choudhary, M., Jones, J.B., and Paret, M.L. 2021. Natural or green synthesis nanomaterials and impact on plant pathogens. In: Balestra, G.M. and Fortunati, E. (eds.), *Nanotechnology-based sustainable alternatives for the management of plant diseases*, Elsevier, pp. 5-29
- Darroudi, M., Ahmad, M.B., Zamiri, R., Zak, A.K., Abdullah, A.H., and Ibrahim, N.A. 2011. Time-dependent effect in green synthesis of silver nanoparticles. *Int. J. nanomed.* 12: 677-681.

- Din, M.I. and Rehan, R. 2017. Synthesis, characterization, and applications of copper nanoparticles. *Anal. Lett.* 50(1): 50-62.
- Din, M.I., Arshad, F., Hussain, Z., and Mukhtar, M. 2017. Green adeptness in the synthesis and stabilization of copper nanoparticles: catalytic, antibacterial, cytotoxicity, and antioxidant activities. *Nanoscale Res. Lett.* 12: 1-15.
- Divte, P.R., Shende, S., Limbalkar, O.M., and Kale, R.A. 2019. Characterization of biosynthesized copper nanoparticle from *Citrus sinensis* and in-vitro evaluation against fungal pathogen *Colletotrichum capsici*. *Int. J. Chem. Stud.* 7:325-330.
- Divya, S., Anusree, A.R., Vigi, S., Jiji, S.G., Das, P.A., Dev, A.R., Thara, S.S., Varghese, E.M., Gopinath, P.P., and Anith, K.N. 2023. Silver nanoparticles green synthesized with leaf extract of disease-resistant tamaranthus genotype effectively suppress leaf blight (*Rhizoctonia solani* Kühn) disease in a susceptible red amaranthus cultivar. *3 Biotech.* 13(6):196-207.
- Dubey, S.P., Lahtinen, M., and Sillanpää, M. 2010. Tansy fruit mediated greener synthesis of silver and gold nanoparticles. *Process Biochem.* 45(7):1065-1071.
- Elemike, E.E., Uzoh, I.M., Onwudiwe, D.C., and Babalola, O.O. 2019. The role of nanotechnology in the fortification of plant nutrients and improvement of crop production. *Appl. Sci.* 9(3): 499.
- El-Naggar, N.E.A., Hussein, M.H., and El-Sawah, A.A. 2017. Bio-fabrication of silver nanoparticles by phycocyanin, characterization, in vitro anticancer activity against breast cancer cell line and in vivo cytotoxicity. *Sci. rep.* 7(1):10844-10857.
- Fardsadegh, B. and Jafarizadeh-Malmiri, H. 2019. Aloe vera leaf extract mediated green synthesis of selenium nanoparticles and assessment of their in vitro antimicrobial activity against spoilage fungi and pathogenic bacterial strains. *Green Process. Synth.* 8(1): 399-407.
- Fu, P.P., Xia, Q., Hwang, H.M., Ray, P.C., and Yu, H. 2014. Mechanisms of nanotoxicity: generation of reactive oxygen species. *J. Food Drug Anal.* 22(1):64-75.
- Gericke, M. and Pinches, A. 2006. Biological synthesis of metal nanoparticles. *Hydrometallurgy*, 83(1-4):132-140.

- Haroon, M., Zaidi, A., Ahmed, B., Rizvi, A., Khan, M.S., and Musarrat, J. 2019. Effective inhibition of phytopathogenic microbes by eco-friendly leaf extract mediated silver nanoparticles (AgNPs). *Indian J. microbial.* 59:273-287.
- Hernández-Díaz, J.A., Garza-García, J.J., Zamudio-Ojeda, A., León-Morales, J.M., López-Velázquez, J.C., and García-Morales, S. 2021. Plant-mediated synthesis of nanoparticles and their antimicrobial activity against phytopathogens. *J. Sci. Food Agric.* 101(4): 270-1287.
- Hossain, A., Abdallah, Y., Ali, M.A., Masum, M.M.I., Li, B., Sun, G., Meng, Y., Wang, Y., and An, Q. 2019. Lemon-fruit-based green synthesis of zinc oxide nanoparticles and titanium dioxide nanoparticles against soft rot bacterial pathogen *Dickeya dadantii*. *Biomedol.* 9(12): 863-875.
- Hossain, A., Hong, X., Ibrahim, E., Li, B., Sun, G., Meng, Y., Wang, Y., and An, Q. 2019. Green synthesis of silver nanoparticles with culture supernatant of bacterium *Pseudomonas rhodesiae* and their antibacterial activity against soft rot pathogen *Dickeya dadantii*. *Molecules*, 24(12): 2303-2318.
- Iravani, S. 2011. Green synthesis of metal nanoparticles using plants. *Green Chem.* 13(10):2638-2650.
- Iravani, S. and Zolfaghari, B. 2013. Green synthesis of silver nanoparticles using *Pinus edulis* bark extract. *Bio. Med. Res. Int.* 13: 115-134.
- Jadoun, S., Arif, R., Jangid, N.K., and Meena, R.K. 2021. Green synthesis of nanoparticles using plant extracts: A review. *Environ. Chem. Lett.* 19:355-374.
- Jamdade, D.A., Rajpali, D., Joshi, K.A., Kitture, R., Kulkarni, A.S., Shinde, V.S., Bellare, J., Babiya, K.R., and Ghosh, S. 2019. *Gnidiaglauca*- and *Plumbago zeylanica*-mediated synthesis of novel copper nanoparticles as promising antidiabetic agents. *Adv. Pharmacol. Pharma. Sci.* 21:226-241.
- Jayaseelan, C., Ramkumar, R., Rahuman, A.A., and Perumal, P. 2013. Green synthesis of gold nanoparticles using seed aqueous extract of *Abelmoschus esculentus* and its antifungal activity. *Ind. Crops Prod.* 45:423-429.

- Jemilugba, O.T., Parani, S., Mavumengwana, V., and Oluwafemi, O.S. 2019. Green synthesis of silver nanoparticles using *Combretum erythrophyllum* leaves and its antibacterial activities. *Colloid Inter. Sci. Commun.* 31:100191-100205.
- Kausar, H., Mehmood, A., Khan, R.T., Ahmad, K.S., Hussain, S., Nawaz, F., Iqbal, M.S., Nasir, M., and Ullah, T.S. 2022. Green synthesis and characterization of copper nanoparticles for investigating their effect on germination and growth of wheat. *Plosone*. 17(6): e0269987.
- Kiruba, D.S.C.G., Vinothini, G., Subramanian, N., Nehru, K., and Sivakumar, M. 2013. Biosynthesis of Cu, Zn, and Ag nanoparticles using *Dodonaea viscosa* extract for antibacterial activity against human pathogens. *J. Nanoparticles*. 15:1-10.
- Kora, A.J., Mounika, J., and Jagadeeshwar, R. 2020. Rice leaf extract synthesized silver nanoparticles: An in vitro fungicidal evaluation against *Rhizoctonia solani*, the causative agent of sheath blight disease in rice. *Fungal Biol.* 124(7): 671-681.
- Kuchibhatla, S.V., Karakoti, A.S., Baer, D.R., Samudrala, S., Engelhard, M.H., Amonette, J.E., Uthasan, S., and Seal, S. 2012. Influence of aging and environment on nanoparticle chemistry: implication to confinement effects in nanocerium. *J. Phys. Chem.* 116(26): 14108-14114.
- Li, Y., Duan, X., Qian, Y., Yang, L., and Liao, H. 1999. Nanocrystalline silver particles: synthesis, agglomeration, and sputtering induced by electron beam. *J. Colloid Interface Sci.* 209(2): 347-349.
- Lotha, R., Shamprasad, B.R., Sundaramoorthy, N.S., Nagarajan, S., and Sivasubramanian, A. 2019. Biogenic phytochemicals (cassinopinin and isoquercetin) capped copper nanoparticles (ISQ/CAS@ CuNPs) inhibits MRSA biofilms. *Microb. Pathog.* 132:178-187.
- Mankad, M., Patil, G., Patel, D., Patel, P., and Patel, A. 2020. Comparative studies of sunlight mediated green synthesis of silver nanoparticles from *Azadirachta indica* leaf extract and its antibacterial effect on *Xanthomonas oryzae*. *Arab. J. Chem.* 13(1):2865-2872.
- Masum, M.M.I., Siddiqua, M.M., Ali, K.A., Zhang, Y., Abdallah, Y., Ibrahim, E., Qiu, W., Yan, C., and Li, B. 2019. Biogenic synthesis of silver nanoparticles using *Phyllanthus*

emblica fruit extract and its inhibitory action against the pathogen *Acidovorax oryzae* strain RS-2 of rice bacterial brown stripe. *Front. Microbiol.* 10:820-834.

Mishra, S., Singh, B.R., Naqvi, A.H., and Singh, H.B. 2017. Potential of biosynthesized silver nanoparticles using *Stenotrophomonas* sp. BHU-S7 (MTCC 5978) for management of soil-borne and foliar phytopathogens. *Sci. rep.* 7(1):45154-45167.

Mishra, V. and Sharma, R. 2015. Green synthesis of zinc oxide nanoparticles using fresh peel extract of *Punica granatum* and its antimicrobial activities. *Int. J. Pharma. Res. Health Sci.* 3(3): 694-699.

Mudunkotuwa, I.A., Pettibone, J.M., and Grassian, V.H. 2012. Environmental implications of nanoparticle leaching in the processing and fate of copper-based nanomaterials. *Environ. Sci. Technol.* 46(13):7001-7010.

Nandhini, M., Rajini, S.B., Udayashankar, A.C., Niranjana, S.R., Lund, O.S., Shetty, H.S., and Prakash, H.S. 2019. Biofabricated zinc oxide nanoparticles as an eco-friendly alternative for growth promotion and management of downy mildew of pearl millet. *Crop Prot.* 121: 103-112.

Noruzi, M. 2015. Biosynthesis of gold nanoparticles using plant extracts. *Bioprocess biosyst. eng.* 38(1): 1-14.

Ogunyemi, S.O., Abdallah, Y., Zhang, M., Fouad, H., Hong, X., Ibrahim, E., Masum, M.M.I., Hossain, A., Mo, J., and Li, B. 2019. Green synthesis of zinc oxide nanoparticles using different plant extracts and their antibacterial activity against *Xanthomonas oryzae pv. oryzae*. *Artif. cells, nanomed. Biotechnol.* 47(1):341-352.

Paulkumar, K., Gnanajobitha, G., Vanaja, M., Rajeshkumar, S., Malarkodi, C., Pandian, K., and Annadurai, G. 2014. *Piper nigrum* leaf and stem assisted green synthesis of silver nanoparticles and evaluation of its antibacterial activity against agricultural plant pathogens. *Sci. World J.* 20: 390-404.

Pham, N.D., Duong, M.M., Le, M.V., and Hoang, H.A. 2019. Preparation and characterization of antifungal colloidal copper nanoparticles and their antifungal activity against *Fusarium oxysporum* and *Phytophthora capsici*. *CR Chim.* 22(11-12):786-793.

- Pirtarighat, S., Ghannadnia, M., and Baghshahi, S. 2019. Green synthesis of silver nanoparticles using the plant extract of *Salvia spinosa* grown in vitro and their antibacterial activity assessment. *J. Nanostructure Chem.* 9: 1-9.
- Rahimi, H. R. and Doostmohammadi, M. 2020. *Nanoparticles synthesis, applications, and toxicity, in Applications of Nanobiotechnology*, (ed.) Stoytcheva, M. and Zlatev, R. IntechOpen, London, pp. 1-16
- Raju, D., Mehta, U. J., and Hazra, S. 2011. Synthesis of gold nanoparticles by various leaf fractions of *Semecarpus anacardium* L. tree. *Trees.* 25: 145-151.
- Savale, A., Ghotekar, S., Pansambal, S., and Pardeshi, O. 2017. Green synthesis of fluorescent CdO nanoparticles using *Leucaena leucocephala* L. extract and their biological activities. *J. Bacteriol. Mycol.* 5(5): 00148-00161.
- Sebeia, N., Jabli, M., and Ghith, A. 2019. Biological synthesis of copper nanoparticles, using Nerium oleander leaves extract: characterization and study of their interaction with organic dyes. *Inorg. Chem. Commun.* 105: 36-46.
- Shah, M., Fawcett, D., Sharma, S., Tripathy, S. K., and Poinern, G. E. J. 2015. Green synthesis of metallic nanoparticles via biological entities. *Mater.* 8(11): 7278-7308.
- Shankar, S. and Rhim, J. W. 2014. Effect of copper salts and reducing agents on characteristics and antimicrobial activity of copper nanoparticles. *Mater. Lett.* 132: 307-311.
- Sharma, P., Pant, S., Dave, V., Tak, K., Sadhu, V., and Reddy, K. R. 2019. Green synthesis and characterization of copper nanoparticles by *Tino sporacardifoliato* produce nature-friendly copper nano-coated fabric and their antimicrobial evaluation. *J. microbiol. methods*, 160: 107-116.
- Shende, S., Gaikwad, N., and Bansod, S. 2016. Synthesis and evaluation of antimicrobial potential of copper nanoparticle against agriculturally important phytopathogens. *Synth.* 1(4): 41-47.
- Some, S., Bulut, O., Biswas, K., Kumar, A., Roy, A., Sen, I. K., Mandal, A., Franco, O. L., Ince, I. A., Neog, K., and Das, S. 2019. Effect of feed supplementation with biosynthesized silver nanoparticles using leaf extract of *Morus indica* L. V1 on *Bombyx mori* L. (Lepidoptera: Bombycidae). *Sci. Rep.* 9(1): 14839-13954.

Subhapiya, S. and Gomathipriya, P.J.M.P. 2018. Green synthesis of titanium dioxide (TiO₂) nanoparticles by *Trigonella foenum-graecum* extract and its antimicrobial properties. *Microb. Pathog.* 116: 215-220.

Uddin, S., Safdar, L.B., Anwar, S., Iqbal, J., Laila, S., Abbasi, B.A., Saif, M.S., Ali, M., Rehman, A., Basit, A., and Wang, Y. 2021. Green synthesis of nickel oxide nanoparticles from *Berberis balochistanica* stem for investigating bioactivities. *Mol.* 26(6): 1548-1562.

Vijayaraghavan, K. and Ashokkumar, T. 2017. Plant-mediated biosynthesis of metallic nanoparticles: A review of literature, factors affecting synthesis, characterization techniques and applications. *J. environ. Chem. Eng.* 5(5): 4866-4883.

Wuithschick, M., Birbaum, A., Witte, S., Sztucki, M., Vainio, U., Pinna, N., Rademann, K., Emmerling, F., Kraehnert, R., and Polte, J. 2015. Turkevich in new robes: key questions answered for the most common gold nanoparticle synthesis. *ACS nano.* 9(7): 7052-7071.

Comment [mj4]: Make according to the format of the journal

UNDER PEER REVIEW

