PLANT SCIENCE TODAY
ISSN 2348-1900 (online)
Vol 11(sp3): 199-209
https://doi.org/10.14719/pst.4606

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RESEARCH ARTICLE

Green synthesis and characterization of CuO nanoparticles derived from *Pimenta dioica* and evaluating its activity against Tobacco streak virus

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OPEN ACCESS

ARTICLE HISTORY
Received: 09 August 2024
Accepted: 09 November 2024
Available online
Version 1.0 : 29 December 2024

Check for updates

Additional information

Peer review: Publisher thanks Sectional Editor and the other anonymous reviewers for their contribution to the peer review of this work.

Reprints & permissions information is available at https://horizonepublishing.com/journals/index.php/PST/open_access_policy

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Indexing: Plant Science Today, published by Horizon e-Publishing Group, is covered by Scopus, Web of Science, BIOSIS Previews, Clarivate Analytics, NAAS, UGC Care, etc
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Shaji H, Kannan R, Harish S, Anita B. Green synthesis and characterization of CuO nanoparticles derived from *Pimenta dioica* and evaluating its activity against *Tobacco streak virus*. Plant Science Today. 2024; 11(sp3): 199-209. https://doi.org/10.14719/pst.4606

Abstract

The synthesis of nanoparticles using plants represents a green and sustainable method that uses natural and non-toxic resources for nanoparticle production. In this study, CuO nanoparticles were synthesized from allspice (*Pimenta dioica*), which served as both the capping and reducing agent during synthesis. Synthesised nanoparticles were validated using UV-Vis spectroscopy, X-ray diffraction, FT-IR analysis and Transmission Electron Microscopy (TEM). The monoclinic phase of the synthesised particles was revealed through the X-ray diffraction pattern indicating its crystalline nature. The UV -visible spectrum showed a strong absorbance of UV rays at a wavelength of 294 nm, confirming the presence of CuO nanoparticles. FT-IR analysis identified different functional groups from the phytochemicals in the plant extract used for the green synthesis, with peaks at 424.34 cm^{-1}, 478.35 cm^{-1}, 555.50 cm^{-1} and 648.08 cm^{-1} corresponding to Cu-O bond vibrations, thereby confirming the existence of CuO nanoparticles. TEM analysis revealed that the nanoparticles had spherical and hexagonal shapes, with sizes ranging from 10 - 20 nm. The antiviral potential of these nanoparticles was assessed against *Tobacco streak virus,* isolated from black gram plants, as spray applications at concentrations of 100, 200, 400 and 500 ppm. The pre-inoculation spray at 500 ppm of CuO nanoparticles, followed by challenge inoculation with the virus, yielded the most effective result, with a reduction of 59.58 % compared to the control. Therefore, the antiviral efficacy of synthesized CuO nanoparticles against plant viruses was established, supporting their potential as a novel strategy for plant management.

Keywords

antiviral; nanoparticles; *Pimenta dioica*; reactive oxygen species; Tobacco streak virus

Introduction

Agriculture is a field of science that focuses on cultivating crops scientifically to address worldwide food demand. Key goals include improving both the quality and quantity of food production and mitigate with the nutritional imbalances. However, abiotic and biotic factors affect reduction in the global food production. Among the biotic factors, pests and diseases play major role in yield loss. Often, it is affected by many diseases leading to substantial losses in yield (1). Plant viruses are sub-microscopic entities that infect the plants which is a production constrains to different crops. Loss in global food production due to plant viral diseases account about 47 % (2). Manage-

ment of plant virus is tedious as it is transmitted mainly by vectors, seeds, planting material, etc. Fungal and bacterial diseases can be managed through different means including chemical fungicides and antibiotics while viral diseases remain particularly challenging to control, lacking effective management strategies. We are expanding the horizon for the management of plant viruses using different methods.

Nanotechnology is a rapidly evolving applied science which make use of particles in nano dimensions (less than 100 nm). Nanotechnology is an applied science which covers multi-dimensional aspect of nanoparticles (3). Nanoparticles is significantly important because of its smaller size and larger surface to volume ratio. Biological synthesis of nanoparticles is an emerging area of green and safe method for metal/metal oxide nanoparticles synthesis using plants and microbes as the reducing and capping agent. The use of plant extract rather than microbes is advantageous due to ease of handling with less biohazard (4). Green synthesis of nanoparticles is safer, ecofriendly, cost-effective approach. This method doesn't require high temperature or pressure, and the process is found to be stable and biocompatible. Thus, green synthesis is a clean, safe and pollution free approach in nanoparticle synthesis (5). Different plant extracts are used for the synthesis of nanoparticles. Various secondary metabolites present in the leaf extracts like terpenoids, flavonoids, alkaloids, etc. reduce the precursor molecule and act as capping agent which stabilize the nanoparticles synthesised.

Allspices (*Pimenta dioica*) is a spices crop belongs to the family Myrtaceae with wide range of application. The essential oil derived from Allspice have typical aroma of clove, nutmeg, pepper and cinnamon. Allspices contain wide range of phenols, polyphenols, flavonoids which impart pharmaceutical importance to the plant species. Centre of origin of Caribbean Island Jamaica. Economic important part of allspices is dried unripe berries. Jamaica is the largest producer of allspices with their exceptionally high quality of essential oil. India is the top exporter of allspices (6). Allspices can be exploited its potential for the reduction of precursor molecule to yield nanoparticles. Allspices leaf extracts were used for the synthesis of gold, copper oxide and silver nanoparticles (7-9).

Green synthesised nanoparticle exhibits wide application including antibacterial, antifungal activity, antioxidant property, etc. Nanoparticle can be used as biofertilizer, nano pesticide, nano based drug delivery system, nano sensor for the detection (10). They exhibit antiviral property against broad spectrum of viruses and reduce the infectivity of viruses. Silver nano particle exhibit antiviral property against potato virus Y and Tomato spotted wilt virus (11). CuO nanoparticle exhibit antiviral property against herpes simplex virus, SARS corona virus (12, 13). However, we are exploring the potential of CuO nanoparticle in the management of plant virus. Tobacco streak virus (TSV) is an important virus infecting cotton, sunflower and many other crop species. Virus is widely expanding its host range of infectivity. TSV cause infection in black gram which cause necrotic lesion and considerable reduction in crop yield. Current study is focused on the synthesis, characterisation of CuO nanoparticle and evaluating the efficacy in reducing *t*TSV infection in black gram.

Materials and Methods

Collection of leaf sample and preparation of leaf extract

P. dioica (allspices) leaves were collected from the tree orchard of Tamil Nadu Agricultural University. The leaves were shade dried for one week and grinded into fine powder. 10 g of leaf powder was dissolved in 100 mL of water and kept in water bath for 30 min at 60 °C. The mixture was then filtered through Whatman filter paper and extract was collected.

Synthesis of nanoparticles

100 mL of 0.01 M copper sulphate solution was prepared, which was titrated against 30 mL allspice leaf extract by continuous stirring for 5 hr at 300 rpm. The pH was adjusted to 8 during stirring. The solution was kept for overnight for the ageing process. The solution was centrifuged to get the pellets. The Pellets were subjected to 3 ethanol washes and 2 distilled water washes. The pellets were dissolved in distilled water and kept in hot air oven to at 60°C to obtain flakes. The flakes were grinded to fine powder and kept in muffle furnace at 600 °C for 2 hr.

Characterization of nanoparticles

The synthesised nanoparticles were characterized through X- Ray Diffraction (XRD), UV-Vis spectroscopy, Fourier-transform infrared spectroscopy (FT-IR), Transmission electron microscopy (TEM) and Energy-dispersive X-ray spectroscopy (EDAX). 100 µg of nanoparticle was sonicated in 100 mL distilled water and the sample was submitted for various characterization process. UV Visible spectroscopy analysis of the nanoparticle in range of 300-600 nm was done using SPECORD 210 PLUS, Germany. FT-IR data was recorded by Shimadzu, Japan which will characterize the functional groups present. XRD results were obtained through Diffractometer Bruker D8 advance indicating the crystalline property of nanoparticles. Size of nanoparticle was determined by TEM analysis using FEI TECHNAI SPIRIT, Netherlands. The elemental composition of nanoparticles was determined by EDAX along with the TEM.

Multiplication and propagation of virus

TSV infected plants were collected from black gram field from Department of Pulses, TNAU, Coimbatore. TSV produce characteristic necrotic lesion in veinal and interveinal regions. The infected samples were collected and inoculated into local lesion host cowpea (CO7) for multiplication and maintenance of the virus. About one gram of infected leaves was macerated with 3 mL sodium phospahte buffer (pH 7.2) in pre-chilled pestle and mortar. Sodium phosphate buffer was prepared using sodium phosphate mono and dibasic, Na_2SO_3, Na_2EDTA and 0.1 % Mercaptoethanol (14). Carborundum powder and celite were used as abrasive. Abrasive was applied before sap inoculation. The sap was applied on the upper surface of leaves and washed well after application. Uninoculated healthy control was

also maintained. Time taken to express the symptoms and nature of symptoms expressed was recorded.

Molecular characterization of virus

Molecular characterization of virus infected samples was performed through Reverse Transcriptase PCR (RT-PCR). Inoculated leaf samples of cowpea and infected green gram plants exhibiting TSV symptoms were used to extract the Total RNA (15). Approximately 100 mg of infected leaf tissue were finely ground with liquid nitrogen and 1 mL of Trizol reagent was mixed with the sample. After incubation for 3 min, it was centrifuged at 10000 rpm for 15 min, followed by separation of aqueous phase containing RNA, mixed with chloroform and centrifuged. The aqueous phase was again transferred and mixed with NaCl and isopropanol solution undergone centrifugation to collect the pellets. Pellets were washed with ethanol, dried and reconstituted in RNase-free water. Non-infected tissue sap served as a control. For first strand cDNA synthesis, 1.5 µg of RNA was combined with random primer (1 µL) and nuclease-free water (6 µL) within a sterile tube. This process utilized the Thermo Scientific RevertAid First Strand cDNA synthesis kit. The thermocycler was set for 5 min at 25 °C followed by 60 min at 42 °C. The reaction mixture was heated to 70 °C for 10 min to stop the reaction.

The synthesized cDNA was PCR amplification using coat protein (CP) gene with the specific primer pair sequence of forward primer 5'AGATAAGTC GCTTCTCGGAC 3' and reverse primer 5' TGCTCGCAT GGGTCATAGAC 3' (16). Reaction conditions were initial denaturation at 94 °C for 2 min, denaturation 94 °C for 1min, annealing 59 °C for 2 min, extension 72 °C for 1 min and final extension 72 °C for 10 min. Reaction conditions of denaturation, annealing and extension was repeated for 30 times. The amplified product undergone agarose gel electrophoresis for illuminating the band formed corresponding to the primer. The amplified PCR product was submitted for sanger sequencing to obtain the nucleotide sequence of virus. CLUSTAL W was used to compare and analyse the consensus sequences of coat protein genes of other known TSV isolates from GenBank. MEGA version 11.0 was used to create the phylogenetic tree at the nucleotide level. Sequences for comparison were retrieved from Genbank. The analysis was made on coat protein sequence with a bootstrap percentage (1000 replication) which was further submitted in the NCBI Genbank database.

Antiviral activity of synthesised CuO nanoparticles

Synthesised nanoparticle was applied in different concentration in cowpea and challenge inoculated with virus. Nanoparticles were dissolved in distilled water and kept in water bath sonicator for 5 min. Different concentrations of nanoparticle at 100, 200, 400 and 500 ppm were applied as pre-inoculation (24 hr before virus inoculation), simultaneous inoculation (along with virus inoculation) and post inoculation (24 hr after virus inoculation). The virus sap was inoculated into the cowpea plant. The observation was taken on 5th day after inoculation of virus.

Results and Discussion

Synthesis of CuO nanoparticle

CuO nanoparticles were obtained by the reduction of precursor molecule ($CuSO_4$ $5H_2O$) using allspices leaf extract. Here leaf extract act as stabilizing and reducing agent. During the reaction, copper ions in the precursor solution react with hydroxide ions to form copper hydroxide molecule

$$Cu^{2+} + 2OH^- \rightarrow Cu(OH)_2(s)$$

Copper hydroxide was then converted to copper oxide (CuO) through a thermal decomposition process (muffling and hot air oven).

$$2Cu(OH)_2(s) \rightarrow 2CuO(s) + 2H_2O(g)$$

While titrating the leaf extract against precursor molecule there was a change in colour of solution after few hours of stirring (Fig. 1). CuO NPs formation was initially confirmed through the change in colour of the reaction

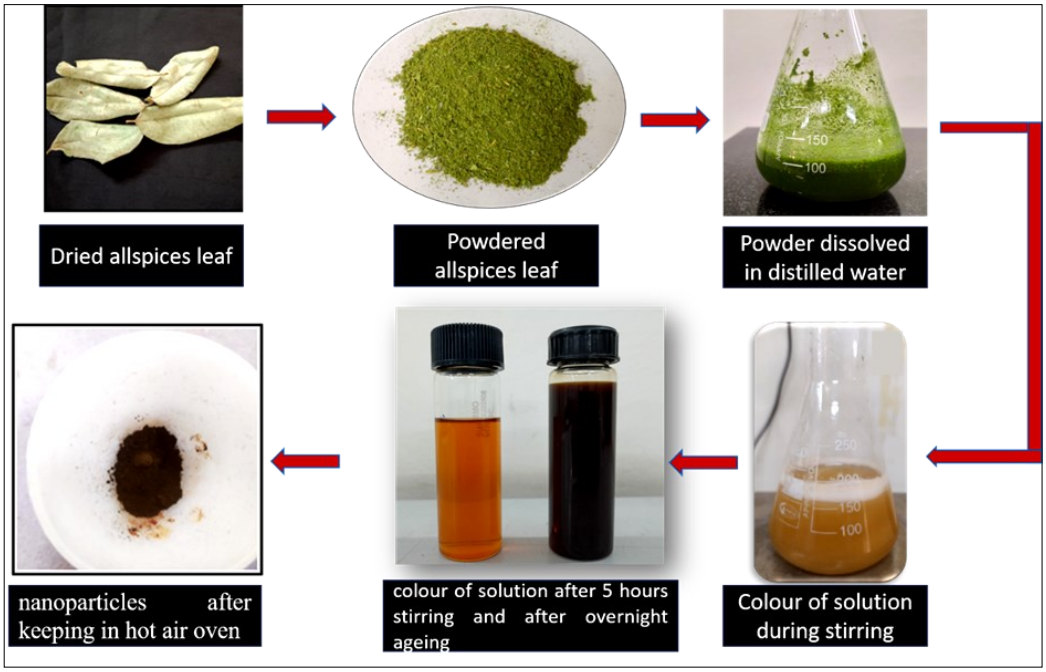


Fig. 1. Flowchart for the synthesis of the nanoparticles.

mixture. This is due to the formation of monoclinic (copper (II) oxide) and cubic (Copper (I) oxide). After keeping the solution for ageing process, the colour of solution again changes to darkish brown from orange-brown. This is due to the complete decomposition of copper hydroxide molecule and the formation of nanoparticles (Fig. 1). Change in the colour of solution is the first indication of nanoparticle formation.

Characterization of synthesised CuO nanoparticles

X-Ray Diffraction

The synthesised particles were further characterized for their nano dimensions using XRD. Crystalline nature of the particle was indicated by the diffraction pattern. 2 Sharp peak in the XRD graph suggests that the strong crystalline nature of nanoparticle synthesised (Fig. 2). XRD pattern of nanoparticles formed diffraction peaks at 2 θ values of (32.74°, 35.81°, 38.96°, 48.87°, 58.27°, 61.69°, 66.41° and 68.56° were assigned to be (110), (002), (111), (202), (020), (202), (113), (311) and (113), which were highly consistent with JCPDS standard no. 01-080-0076 which has monoclinic phase CuO NPs (17, 18). The diffraction peak of nanoparticles coincides with the standard CuO nanoparticles pattern indicating the crystalline nature of CuO nanoparticles which is monoclinic (copper (II) oxide).

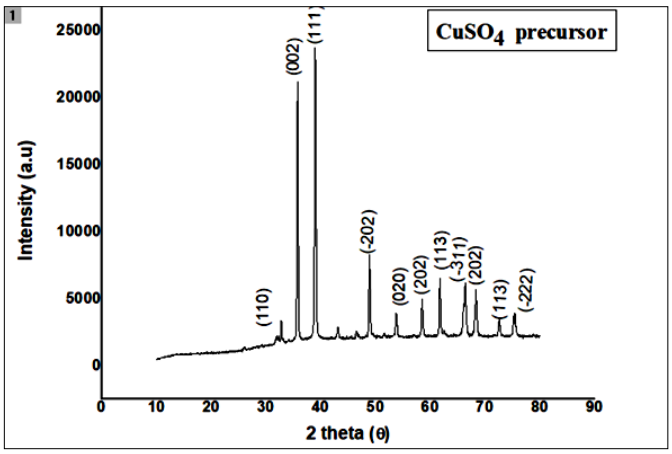


Fig. 2. XRD diffraction peak of synthesised CuO nanoparticle.

UV- Visible spectroscopy

The UV spectrum of the CuO nanoparticles was analysed by dispersing it in distilled water and subjected to spectral analysis. The spectrum exhibits strong absorbance of UV rays within the 294 nm wavelength indicating the presence of CuO nanoparticles in the sample (Fig. 3). It was reported that the UV spectrum of the CuO nanoparticles synthesised dispersed in water exhibited a strong absorbance of UV rays between the wavelengths of 200-300 nm (19). The peak observed at 294 nm was attributed to the surface plasmon absorption of CuO nanoparticles. According to a study, this phenomenon happens when incoming UV radiation causes the free conduction band electrons within the metal oxide (CuO) to oscillate coherently (20). Surface plasmon absorption often occurs when the incident light's wavelength is substantially longer than the nanoparticle's diameter.

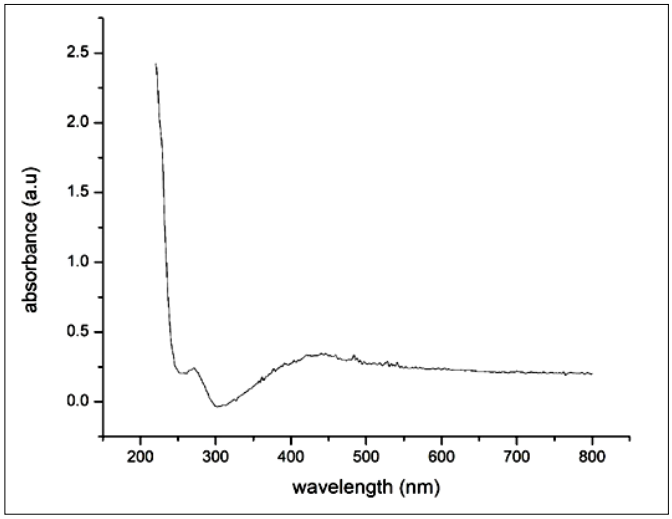


Fig. 3. UV- Visible image of CuO nanoparticle synthesised from allspices leaf extract.

Fourier Transform Infrared Spectroscopy (FT-IR)

FT-IR used to identify functional groups of chemicals in plant extracts utilised for synthesis of CuO nanoparticles (Fig. 4). Peaks at 424.34 cm^{-1}, 478.35 cm^{-1}, 555.50 cm^{-1} and 648.08 cm^{-1} is due to Cu-O bond vibration indicating the presence of synthesised CuO nanoparticles. Similar peaks

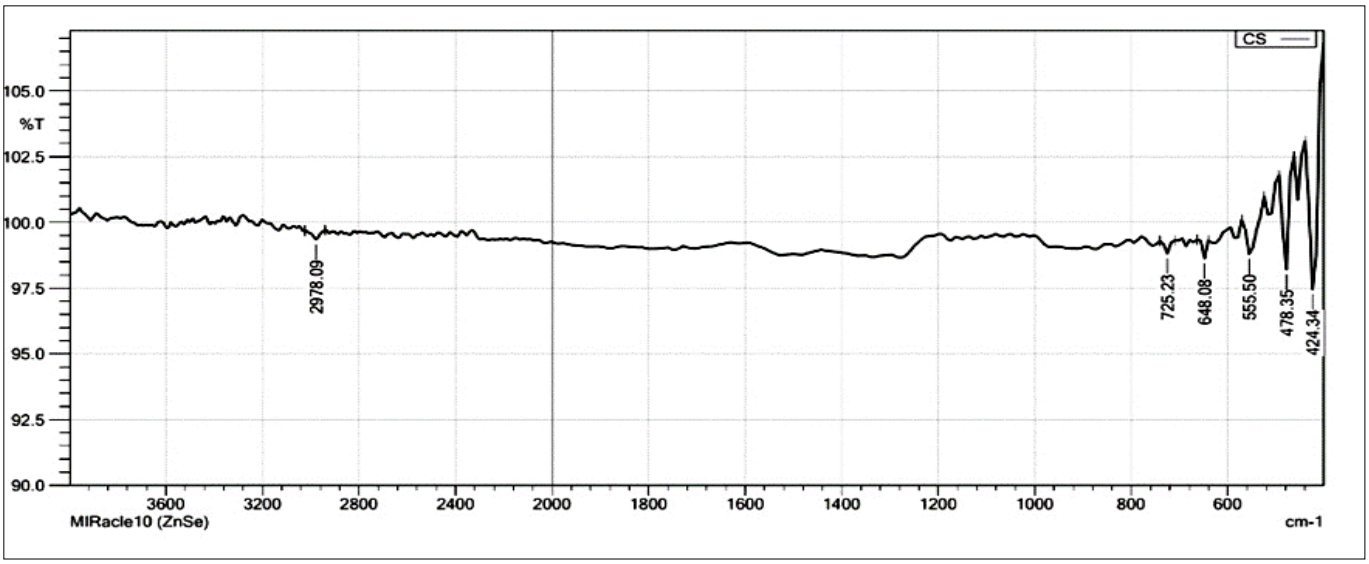


Fig. 4. FT-IR spectral analysis of CuO nanoparticles.

were observed at 488 and 658 cm^{-1} in the spectrum of green synthesised CuO nanoparticles (21). Peaks ranging 725.23 attributed to presence of phenolic groups and alcohol, C–N stretching in amines. The peak formed at 2978.09 cm^{-1} indicated the formation of C-H stretching (435 cm^{-1}). FT-IR spectral peak confirmed the existence of several compounds, including alkynes, aromatics, alkane compounds, alkyl halides, nitro compounds and different aroma-related phytocompounds. These active plant compounds likely played a role in reducing and capping agent for CuO nanoparticles. The main constituent of allspices is eugenol, methyl eugenol which contain alcoholic and phenolic compounds (22). C-H bond stretching is contributed by eugenol and other constituent of allspices.

Transmission Electron Microscope (TEM)

TEM image reveals the actual size and shape of nanoparticles synthesized. The CuO nanoparticle synthesized have characteristic hexagonal shape and spherical shape (Fig. 5). The average size of CuO nanoparticles is 10-20 nm. Nanoparticle can acquire various shaped depending on synthesis procedure. Different metal atoms can exhibit hexagonal structure depending on reaction conditions. CuO nanoparticle can exhibit hexagonal shape. It was also synthesized nanoparticles CuO nanoparticles which were hexagonal in shape with size ranging from 10-20 nm (23).

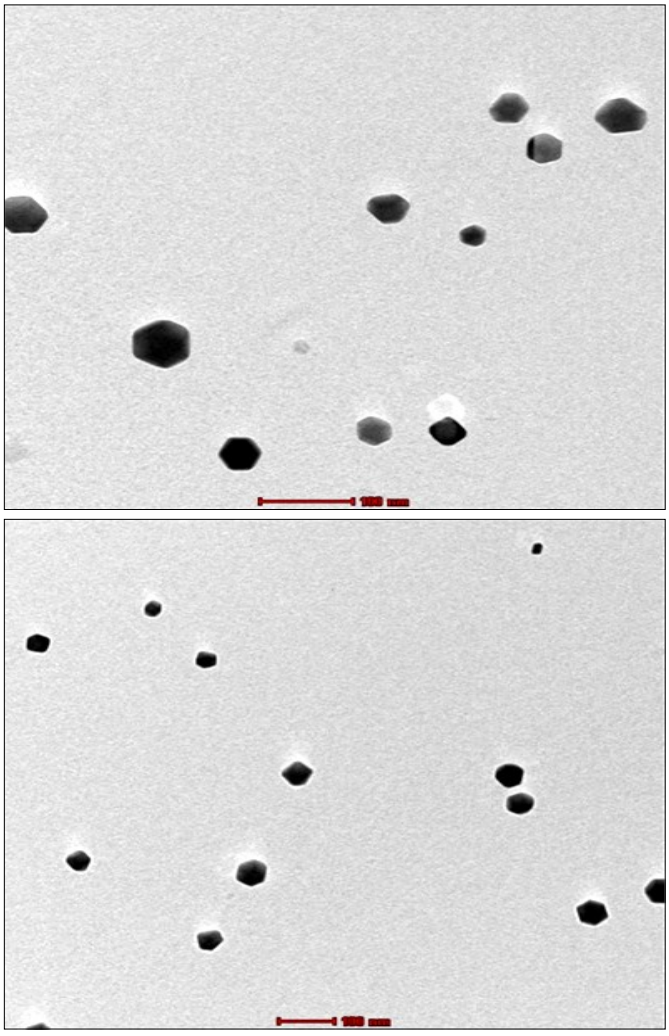
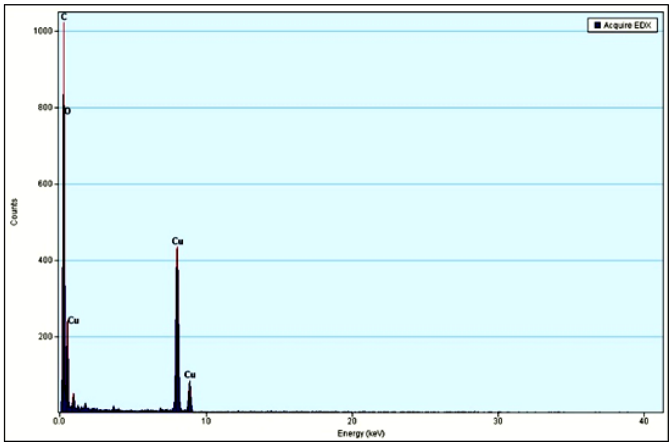


Fig. 5. TEM image of CuO nanoparticles.

Energy-dispersive X-ray spectroscopy (EDAX)

The EDAX profile showed the presence of Cu as elemental composition of green synthesized CuO nanoparticles. The highest peak of O indicates the presence oxide form of Cu nanoparticle (Fig. 6). This confirms the synthesised nanoparticle is of CuO material.



Multiplication and propagation of virus

Symptomatology of Tobacco streak virus

TSV infection in black gram plants appear as necrotic lesion around veinal region which later coalesce and results in complete drying of the leaf. Necrotic lesion starts from one part of leaf lamina later spread to other leaf parts. Undersurface of the leaves also contain interveinal necrosis. Necrosis spread to petiole and stem region and ultimately

Fig. 7. Black gram field infected by Tobacco streak virus.

lead to the drying of plants (Fig. 7). It describes the symptoms of TSV in black gram plant as necrotic spots which correlates with the symptoms described (24). TSV from infected plant samples was mechanically inoculated into the local lesion host (cowpea CO7) which produces symptoms after 3 days of inoculation. Different symptoms were produced on TSV inoculated cowpea plant. On the fourth day after inoculation, round chlorotic spots appeared on the cotyledonary leaves later changes into necrotic spots (Fig. 8). Additionally, systemic infection is produced on subsequent leaves which include veinal necrosis, stem necrosis, petiolar necrosis. Infected plant eventually dies within 10-12 days under greenhouse conditions (Fig. 9). TSV obtained from various sources, including sunflower (25), groundnut (26), soybean and black gram (27), which was mechanically inoculated onto cowpea plants.

Fig. 8. Necrotic spots appeared on cowpea plant on inoculation with TSV. Chlorotic spots produced on cowpea leaf on virus inoculation.

Fig. 9. Veinal necrosis and petiolar necrosis on TSV inoculated cowpea plants.

Molecular characterization of Tobacco streak virus

cDNA synthesised from RNA of TSV infected samples were subjected to PCR amplification using coat protein (CP) specific primer. Field samples and local lesion samples were amplified producing a band at expected amplicon size of approximately 750 bp size (Fig. 10). It was employed Reverse Transcriptase PCR (RT-PCR) as a rapid and dependable method to detect TSV in various crops, including cotton, sunflower, urd bean and soybean (28). They utilized specific primers targeting the coat protein gene, resulting in the amplification of a region approximately 750

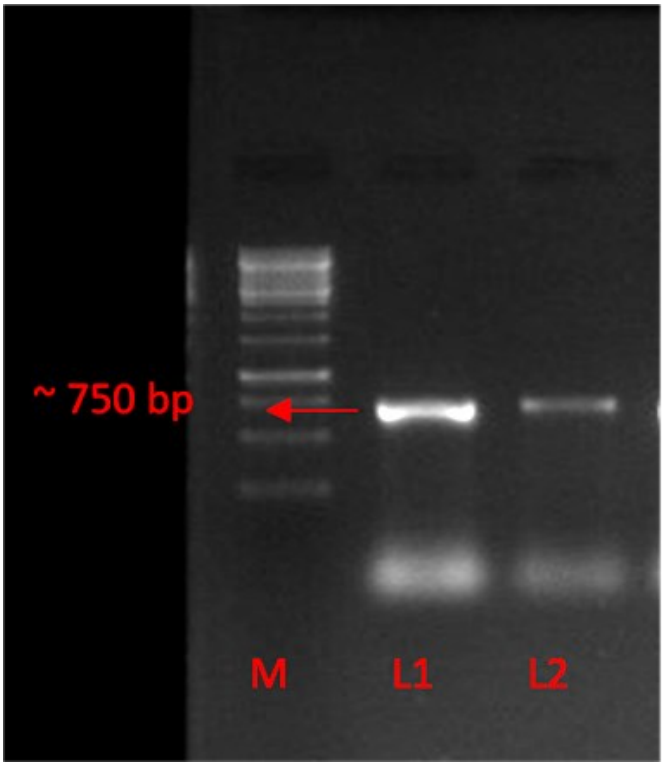


Fig. 10. PCR amplification of TSV infected green gram sample and local lesion sample.

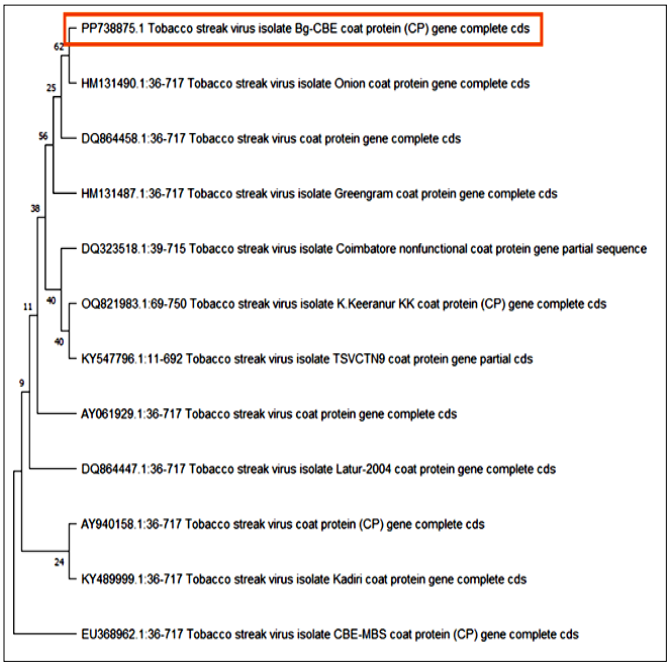


Fig. 11. Phylogenetic tree of Tobacco streak virus.

base pairs in size for TSV detection. Amplified PCR product was given for sanger sequencing and obtained the partial sequence. The sequence was submitted in NCBI site and obtained accession number as for nulcleotide sequence with coat protein id XAH03903.1. The sequence (PP738875.1) was compared with corresponding sequence of known TSV isolates at amino acid and nucleotide sequence levels. Black gram TSV show maximum percent identity with TSV isolates from onion (99.86 %), sunflower (99.58 %) (AY061929.1, HM131490.1) (Fig. 11). The amino acid sequence of TSV isolate show maximum percent identity with TSV isolates from sunflower (99.58 %) and green gram (99.16 %) (AAL31701.1, ADI99750.1). This confirms the presence of TSV in infected plant samples.

Antiviral property of synthesised CuO nanoparticles

The antiviral efficacy of synthesized CuO nanoparticles was evaluated against TSV. Different concentration of CuO nanoparticles was sprayed on cowpea and challenge inoculated with virus at different time intervals and the number of lesions produced was counted (Table 1). The number of lesions produced was less at 500 ppm when applied in plant as pre inoculation spray that produced 8.33 lesions per plant (Fig. 12). Percent reduction of virus over control was found high when applied at 500 ppm which produced 59.58 % reduction followed by 200 ppm of 48.25 % with 10.67 lesions per plant. Highest number of lesions was produced in inoculated control plant with 20.61 lesions per cowpea plant. In simultaneous inoculation of nanoparticles, 10.33 lesions per plant were observed at 500 ppm concentration followed by 200 ppm with 13.17 lesions per plant. Application of 500 ppm recorded 49.86 % reduction over the control found to be highest followed by 200 ppm (36.12 %). Simultaneous inoculation of nanoparticles at 100 ppm found the highest number of lesions with 21.17 which is found to be higher than control. (-2.7 % reduction over the control). Post inoculation treatment of nanoparticles also reduced the virus concentration. Application at 200 ppm, 400 ppm and 500 ppm reduced the number of lesions over control by 12.28, 12.17 and 13.17 with reduction of 40.43 %, 40.97 % and 36.12 % respectively. Post inoculation at 100 ppm concentration of nanoparticles was found ineffective which produced 23.67 lesions per plant which is 14.83 % higher than control.

Pre inoculation spray nanoparticle produced significant reduction in virus titre compared to post and simultaneous spray of nanoparticles. Spraying of nanoparticles

Table 1. Number of lesions and per cent reduction in number of lesions produced in cowpea treated with nanoparticles and challenge inoculated with virus

Treatment	Pre- inoculation spray	Reduction over control (%)	Simultaneous spray	Reduction over control (%)	Post- inoculation spray	Reduction over control (%)
100 ppm	12.70^{ab} (20.88)	38.38	21.17^{c} (27.39)	-2.70	23.67^{b} (29.11)	-14.83
200 ppm	10.67^{b} (19.06)	48.25	13.17^{ab} (21.28)	36.12	12.28^{a} (20.51)	40.43
400 ppm	12.50^{b} (20.70)	39.35	15.83^{b} (23.45)	23.18	12.17^{a} (20.41)	40.97
500 ppm	8.33^{a} (16.78)	59.58	10.33^{a} (18.75)	49.86	13.17^{a} (21.28)	36.12
Control	20.61^{c} (27)		20.61^{c} (27)		20.61^{b} (27)	
CD	3.8381		3.6562		3.7581	
SE(d)	1.7225		1.6409		1.6867	

Fig. 12. Reduction in number of lesions produced by nanoparticle in different concentration in cowpea.

even after infection of virus also reduced the virus titre to an extent. It was studied about the impact of CuO nanostructures towards the management of *Zucchini yellow mosaic virus* (ZYMV) in squash) (29). The nanostructure controls the virus by reducing the disease severity by 85 %. He explained that copper oxide nanoparticles effectively limit ZYMV viral infectivity by obstructing virus-cell contact, potentially influenced by nanoparticle size and zeta potential. They induce resistance-related gene expression, leading to PR protein production that blocks virus movement through plasmodesmata. They may also activate PR genes and stress indicators in plants.

The CuO nanoparticles may bind with virus and inhibit virus replication in host plants, effectively inactivating the virus. CuO nanoparticles were synthesized using *Syzygium alternifolium* fruit extract and assess their effectiveness in combating Newcastle Disease Virus (NDV) (30). He reported that the nanoparticle can control virus effectively control viruses. It was synthesised ZnO nanoparticles from *Menta spicata* and evaluated its antiviral potential against *Tobacco mosaic virus* (TMV) (31, 32). He also reported that double foliar application of ZnO nanoparticles, was the most effective treatment by 90.21 % reduction of viral accumulation at 24 hr before and 24 hr after TMV-inoculation. Nanoparticle application also induce PAL, PR-1 genes at the transcriptional levels indicating the induced plant systemic resistance. Antiviral property of various metal and metal oxide nanoparticles was evaluated against different plant viruses (33-35).

Mode of action of virus particles

Nanoparticles has been widely exploited as the novel agent for the management of virus particles. Ag nanoparticle has been used for the management of plant viruses like *Tomato spotted wilt virus, Potato virus X, Tobacco mosaic virus,* etc. (35, 36). Interaction of nanoparticles with viral particle occur directly and indirectly. Application of nanoparticles will induce the systemic acquired resistance (SAR) against the viruses and increase the levels of total soluble proteins (TSP), antioxidant enzymes polyphenol oxidase (PPO) and peroxidase (POD) activities. Nanoparticle can also interfere with the replication cycle of virus and affect the replication cycle. Even it will affect the transmission and encapsidation process of viral virions (37). The nanoparticles could potentially affect various stages of viral activity, including fusion, binding, infectivity and replication. They generate reactive oxygen species (ROS) and protein oxidation, rendering the virus inactive (Fig. 13). Additionally, the NPs might disrupt virus recognition and entry into host plants. By interacting with glycoprotein receptors of virus surface proteins, they hinder the virus recognition in host cells.

Conclusion

This study explored the potential of allspice-derived CuO nanoparticles for controlling plant viruses, using copper sulfate as a precursor and leaf extract as a reducing agent. Characterization of the nanoparticles showed crystalline CuO particles with a hexagonal shape and an average size of 20 nm, confirmed by XRD, UV-Vis, FT-IR and TEM analysis. The synthesized nanoparticles were tested against TSV, a pathogen affecting crops like sunflower, cotton and pulses. When applied before or after virus inoculation, the nanoparticles reduced viral titre, with the most significant effect (59.58 % reduction) observed when applied at 500 ppm before inoculation. The nanoparticles likely inhibit viral replication by binding to the coat protein and nucleic acids, while also inducing systemic acquired resistance (SAR) in plants. The study concludes that green-

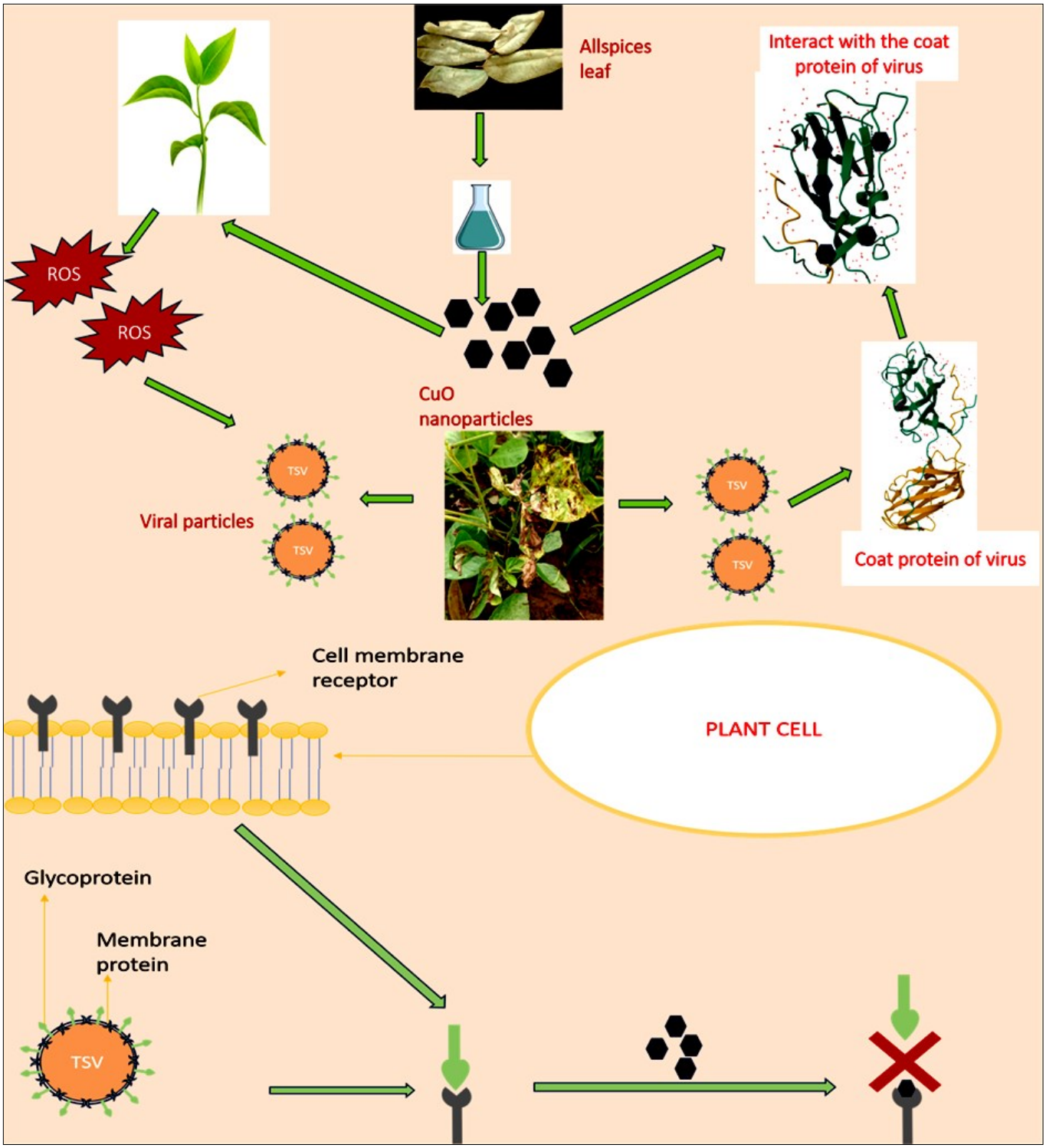


Fig. 13. Mechanism of action of nanoparticles on viral particles. Nanoparticle will produce Reactive Oxygen Species (ROS) in cowpea plant which will affect viral activity. Nanoparticle will interact with coat protein of virus and inhibit the viral multiplication. The nanoparticle inhibits the interaction between glycoprotein of *Tobacco streak virus* with cell membrane receptor which prevent the recognition of viral protein with host cell receptors.

synthesized CuO nanoparticles possess promising antiviral activity against plant viruses. The nanoparticles when encapsulated with a biopolymer to increase the efficiency of application. The nanoparticle formation can be applied in field level to reduce the virus infection in black gram and green gram field.

Acknowledgements

The authors will be thankful to UPL Pvt. Ltd (F37ANQ) for the financial support provided, Department of Plant Pathology and Centre for Agricultural Nanotechnology for facilitating the technical support towards the completion of the research work. The authors are thankful to Dr. S. Thirumalairajan, Ramalingaswami Re-entry Faculty, Department of Nano Science and Technology, TNAU, Coimbatore, India for the technical support provided.

Authors' contributions

HS provided basic idea of work and complete research work was done under the guidance of RK. HS and RK contributed for the preparation of manuscript. Both authors read and approved the final manuscript. SH participated in

the design of the study and performed the statistical analysis. BA contributed to the characterization part of nanoparticles. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None.

References

1. Dutta P, Kumari A, Mahanta M, Biswas KK, Dudkiewicz A, Thakuria D, et al. Advances in nanotechnology as a potential alternative for plant viral disease management. Front Microbiol. 2022;13:935193. https://doi.org/10.3389/fmicb.2022.935193
2. Boualem A, Dogimont C, Bendahmane A. The battle for survival between viruses and their host plants. Curr Opin Virol. 2016;17:32–38. https://doi.org/10.1016/j.coviro.2015.12.001
3. Dauthal P, Mukhopadhyay M. Noble metal nanoparticles: plant-mediated synthesis, mechanistic aspects of synthesis and applications. Ind Eng Chem Res. 2016;55(36):9557–77. https://doi.org/10.1021/acs.iecr.6b00861
4. Annu AA, Ahmed S. Green synthesis of metal, metal oxide nanoparticles and their various applications. In: Handbook of Eco-materials. Springer International Publishing; 2018.p.1–45 https://doi.org/10.1007/978-3-319-48281-1_115-1
5. AnsariMA, Alzohairy MA. One-pot facile green synthesis of silver nanoparticles using seed extract of *Phoenix dactylifera* and their bactericidal potential against MRSA. Evid Based Complement Alternat Med.2018;(1):1860280. https://doi.org/10.1155/2018/1860280
6. Zhang L, Lokeshwar BL. Medicinal properties of the Jamaican pepper plant *Pimenta dioica* and Allspice. Curr Drug Targets. 2012;13(14):1900–06. https://doi.org/10.2174/138945012804545641
7. Fadaka A, Aluko O, Awawu S, Theledi K. Green synthesis of gold nanoparticles using *Pimenta dioica* leaves aqueous extract and their application as photocatalyst, antioxidant and antibacterial agents. J Multidiscip Appl Nat Sci. 2021;1(2):78–88 https://doi.org/10.47352/jmans.v1i2.81
8. Geetha AR, George E, Srinivasan A, Shaik J. Optimization of green synthesis of silver nanoparticles from leaf extracts of *Pimenta dioica* (Allspice). The Scientific World J. 2013(1):362890 https://doi.org/10.1155/2013/362890
9. Pillai RR, Sreelekshmi PB, Meera AP. Enhanced biological performance of green synthesized copper oxide nanoparticles using *Pimenta dioica* leaf extract. Mater Today Proc. 2022;50:163–72. https://doi.org/10.1016/j.matpr.2021.11.547
10. Rai PK, Kumar V, Lee S, Raza N, Kim KH, Ok YS, et al. Nanoparticle-plant interaction: Implications in energy, environment and agriculture. Environ Int. 2018;119:1–9. https://doi.org/10.1016/j.matpr.2021.11.547
11. Shafie RM, Salama AM, Farroh KY. Silver nanoparticles activity against Tomato spotted wilt virus. Middle East J Agric Res. 2018;7:1251–67.
12. Merkl P, Long S, McInerney GM, Sotiriou GA. Antiviral activity of silver, copper oxide and zinc oxide nanoparticle coatings against SARS-CoV-2. Nanomaterials. 2021;11(5):1312. https://doi.org/10.3390/nano11051312
13. Tavakoli A, Hashemzadeh MS. Inhibition of herpes simplex virus type 1 by copper oxide nanoparticles. J Virol Methods. 2020;275:113688. https://doi.org/10.1016/j.jviromet.2019.113688
14. Vinodkumar S, Nakkeeran S, Renukadevi P, Mohankumar S. Antiviral activity of the consortia of *Bacillus* spp. and phyto-antiviral principles towards the management of tobacco streak virus (TSV) in cotton. J Mycol Plant Pathol. 2017;47(4):355–68.
15. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem. 1987;162(1):156–59. https://doi.org/10.1016/0003-2697(87)90021-2
16. Rajamanickam S, Karthikeyan G. Sequence diversity analysis of Tobacco streak virus infecting okra (*Abelmoschus esculentus* L.) in India. J Pure Appl Microbiol. 2014;8:4759–67.
17. Kumar A, Saxena A, De A, Shankar R, Mozumdar S. Facile synthesis of size-tunable copper and copper oxide nanoparticles using reverse microemulsions. RSC Adv. 2013;3(15):5015–21. https://doi.org/10.1039/C3RA23455J
18. Suleiman M, Mousa M, Hussein A, Hammouti B, Hadda TB, Warad I. Copper (II)-oxide nanostructures: synthesis, characterizations and their applications-review. J Mater Environ Sci. 2013;4(5):792–97.
19. Rangasamy M, Gopal SK, Indhumathi A, Loganathan S, Manikandan S, Naresh R. Green synthesis and characterization of copper oxide nanoparticles using *Tecoma stans*. J Pharm Res Int. 2023;35(7):9–16. https://doi.org/10.9734/jpri/2023/v35i77335
20. Altikatoglu M, Attar A, Erci F, Cristache CM, Isildak I. Green synthesis of copper oxide nanoparticles using *Ocimum basilicum* extract and their antibacterial activity. Fresenius Environ Bull. 2017;25(12):7832–37.
21. Vishveshvar K, Krishnan MVA, Haribabu K, Vishnuprasad S. Green synthesis of copper oxide nanoparticles using *Ixiro coccinea* plant leaves and its characterization. BioNanoScience. 2018;8:554–58. https://doi.org/10.1007/s12668-018-0508-5
22. Charles DJ. Allspice. In: Antioxidant properties of spices, herbs and other sources. New York: Springer; 2013. p.145–50. https://doi.org/10.1007/978-1-4614-4310-0_6
23. Umar A, Akhtar MS, Dar GN, Baskoutas S. Low-temperature synthesis of α-Fe_2O_3 hexagonal nanoparticles for environmental remediation and smart sensor applications. Talanta. 2013;116:1060–66. https://doi.org/10.1016/j.talanta.2013.08.026
24. Sumalatha V, Ayodhya D, Balchander V. Facile synthesis of hexagonal-shaped CuO NPs from Cu (II)-Schiff base complex for enhanced visible-light-driven degradation of dyes and antimicrobial studies. Inorg Chim Acta. 2023;548:121358. https://doi.org/10.1016/j.ica.2022.121358
25. Natarajan S, Kannan R, Ramamoorthy V, Sankareswari RU. *In vitro* evaluation of different plant stages of black gram to assess the susceptibility by Tobacco streak virus. Int J Chem Stud. 2018;6:1550–53.
26. Ramiah M, Bhat AI, Jain RK, Pant RP, Ahlawat YS, Prabhakar K, et al. Isolation of an isometric virus causing sunflower necrosis disease in India. Plant Dis. 2001;85(4):443. https://doi.org/10.1094/PDIS.2001.85.4.443B
27. Reddy AS, Prasada Rao RDVJ, Thirumala-Devi K, Reddy SV, Mayo MA, Roberts I, et al. Occurrence of Tobacco streak virus on peanut (*Arachis hypogaea*) in India. Plant Dis. 2002;86(2):173–78. https://doi.org/10.1094/PDIS.2002.86.2.173
28. Ladhalakshmi D, Ramiah M, Ganapathy T, Krishna Reddy M, Salaheddin K, Merin B, et al. First report of the natural occurrence of Tobacco streak virus on black gram (*Vigna mungo*). Plant Pathol. 2006;55(4). https://doi.org/10.1111/j.1365-3059.2006.01364
29. Bhat AI, Jain RK, Chaudhary V, Reddy MK, Ramiah M, Chattannavar SN, et al. Sequence conservation in the coat protein

gene of tobacco streak virus isolates causing necrosis disease in cotton, mung bean, sunflower and sunn-hemp in India. Ind J Biotech. 2002;1(4):350–56.

30. Derbalah A, Abdelsalam I, Behiry SI, Abdelkhalek A, Abdelfatah M, Ismail S, et al. Copper oxide nanostructures as a potential method for control of zucchini yellow mosaic virus in squash. Pest Manage Sci. 2022;78(8):3587–95. https://doi.org/10.1002/ps.7001

31. Yugandhar P, Vasavi T, Uma Maheswari Devi P, Savithramma N. Bioinspired green synthesis of copper oxide nanoparticles from *Syzygium alternifolium* (Wt.) Walp: characterization and evaluation of its synergistic antimicrobial and anticancer activity. Appl Sci. 2017;(7):417–27. https://doi.org/10.1007/s13204-017-0584-9

32. Abdelkhalek A, Al-Askar AA. Green synthesized ZnO nanoparticles mediated by *Mentha spicata* extract induce plant systemic resistance against Tobacco mosaic virus. Appl Sci. 2020;10 (15):5054. https://doi.org/10.3390/app10155054

33. Al-Askar AA, Aseel DG, El-Gendi H, Sobhy S, Samy MA, Hamdy E, et al. Antiviral activity of biosynthesized silver nanoparticles from pomegranate (*Punica granatum* L.) peel extract against Tobacco mosaic virus. Plants. 2023;12(11):2103. https://doi.org/10.3390/plants12112103

34. Alkubaisi NA, Aref NM. Dispersed gold nanoparticles potentially ruin gold barley yellow dwarf virus and eliminate virus infectivity hazards. Appl Nanosci. 2017;7:31–40. https://doi.org/10.1007/s13204-016-0540-0

35. Mahfouze HA, El-Dougdoug NK, Mahfouze SA. Virucidal activity of silver nanoparticles against Banana bunchy top virus (BBTV) in banana plants. Bull Natl Res Cent. 2020;44:1–1. https://doi.org/10.1186/s42269-020-00433-6

36. Noha K, Bondok AM, El-Dougdoug KA. Evaluation of silver nanoparticles as antiviral agent against ToMV and PVY in tomato plants. Sciences. 2018;8(01):100–11.

37. Cai L, Jia H, Liu C, Wang D, Sun X. Foliar exposure of Fe_3O_4 nanoparticles on *Nicotiana benthamiana*: Evidence for nanoparticles uptake, plant growth promoter and defense response elicitor against plant virus. J Hazard Mater. 2020;393:122415. https://doi.org/10.1016/j.jhazmat.2020.122415