"DEVELOPMENT OF A GRAPHENE NANOMATERIAL-BASED SOLUTION FOR DEACTIVATING TOBACCO MOSAIC VIRUS IN SOLANUM LYCOPERSICUM"

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Abstract

The green synthesis of graphene nanoparticles by leaf extract and precursor graphene nanomaterial These nonmaterial effects on tomato seed germination and viral deactivation The TMV virus poses a major challenge to tomato production in numerous countries, resulting in extensive harm to tomato plants and substantial global losses. The is a highly infectious plant virus that affects a wide range of crops, including *Solanum lycopersicum.* Current methods of controlling TMV are limited and often involve the use of harmful chemicals that can negatively impact the environment, animal health, and human health. In this study, we propose the use of graphene nanomaterials as a potential solution for deactivating TMV in *Solanum lycopersicum*. Graphene nanoparticles were synthesised using a modified by hummers method and characterised using UV-visible spectroscopy. this method to quantify the nanoparticles' optical characteristics and absorbance properties. The graphene nanomaterial-based solution was effective in deactivating TMV in *Solanum lycopersicum* plants, with a significant reduction in TMV levels observed in treated plants compared to untreated controls. The graphene nanomaterials were found to be non-toxic to the plants, with a positive impact observed on plant growth and development as they promoted seedling germination rate and enhanced root and shoot length. This study provides a foundation for future research on the various applications of graphene nanomaterials in agriculture and the environment. Further research can explore their potential benefits and risks, as well as optimise their production and application methods to ensure their safe and sustainable use. These findings suggest that graphene nanomaterials may be a promising solution for controlling TMV in *Solanum lycopersicum* and other crops, with potential applications in sustainable agriculture. Further studies are needed to optimise the concentration and application of the graphene nanomaterials as well as evaluate their long-term effects on plant health and the environment.

Keywords: Graphene nanoparticles, Tomato seed germination, TMV virus deactivation, Plant growth enhancer, Antiviral agent, Agriculture.

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1.Introduction

The green synthesis of graphene nanoparticles typically involves the use of natural precursors, such as plant extracts, This approach offers several advantages over conventional synthesis methods, such as the use of toxic chemicals and high temperatures and pressures, which can lead to environmental pollution and health hazards. Nanotechnology is a promising alternative to synthetic pesticides in agriculture, known as nanoagriculture. It involves the use of nanosized materials to enhance crop productivity and reduce the need for pesticides. (Jaiswal, A. Et al.,2021). Plant viruses are a pervasive and global problem, causing significant damage to various types of plants. These viruses are responsible for substantial

reductions in both crop quality and yield, with virus diseases accounting for a staggering 47% of the 15% of worldwide food manufacturing lost due to plant diseases (Boualem and Anderson et al., 2004). These losses can be attributed to physiological, molecular, and biochemical changes that occur in plants infected with Tomato production is particularly affected by virus diseases, with *Tobacco mosaic virus* and *Potato virus Y"* being two of the most widespread and damaging viruses in warm climates. These viruses can cause severe damage to tomato plants and result in significant reductions in yield (Binyam, 2015). This has led to significant challenges in tomato production in many countries. This technology has the potential to increase food production, improve food quality, and protect plants from pathogens. (Vaghasiya et al., 2022). "Nanoparticles are in the 1100 nm range and can be classified as metallic or nonmetallic. They possess unique properties such as optical activity, high surface area, mechanical strength, and chemical reactivity, which make them valuable in various applications. "Nanoparticles have diverse applications in agriculture, including as animal feed supplements, crop micronutrients, herbicides, water purifiers, and sewage treatment agents." Aluminium nanoparticles and graphene nanoparticles have various benefits in agriculture, serving as both antimicrobial agents and plant growth promoters. (Vilardi, 2020) Nanoparticles, when used at the right dosage, can promote plant growth and inhibit viral infections. The mechanisms behind their antiviral properties are not well understood. Silver nanoparticles have been shown in some studies to prevent the growth of various plant viruses, including *Bean yellow mosaic virus, Tomato mosaic virus*, and *potato virus Y*, by binding to the virus particles and preventing their nucleic acid replication. The use of silver NPs can induce systemic acquired resistance (SAR) and improve the release of ROS, which are linked to increased antioxidant activity. Viruses are harmful parasites that infect host cells and cause significant physiological impairments, such as necrosis, stunting, and even the death of the host plant. In agriculture, virus infection poses a significant threat to crop production and can cause symptoms such as mosaic, mottling, and malformation of fruits. (Lin Cai et al. 2019). Tomatoes are an important economic crop, but virus-induced diseases have led to reduced yield and quality, causing significant losses. TMV is a member of the Virgaviridae family and includes economically damaging viruses like the *Tobacco mosaic virus,* which infects tomatoes everywhere. ToMV is a widespread virus that infects tomatoes globally, causing significant losses in production. (Sofy et al., 2021). Pesticides have been developed and widely used to protect crops and plants from various pathogens, including fungi, bacteria, and viruses. They work by either killing or inhibiting the growth of these pathogens, which in turn helps to prevent damage to crops and ensure better yields. However, the use of pesticides has also been associated with negative environmental impacts and health risks, which has led to increasing interest in alternative methods such as biological control and nanotechnology. (Wu et al., 2016). Almunium nanoparticles play a vital role in plant processes like photosynthesis and chlorophyll production. (Iannone et al., 2021), and graphene matter act as plant growth promoters without causing any negative effects on the growth and development of tomato plants. (Ashraf et al., 2022). Nanotechnology provides an alternative solution for managing plant pathogens, as traditional methods can be harmful to the ecosystem and slow. Organic fungicides and biocontrol agents are also available but have limitations. Nanofungicides such as Ag NPs, Cu
NPs, TiO2, chitosan nanoparticles, graphene, and

Nanoparticles such as zinc and silica nanoparticles have shown promise in controlling virus infection in crops, particularly TMV, both in vitro and in vivo. These nanoparticles could potentially serve as a new antiviral strategy in agriculture and address the significant threat to human food security posed by virus infections in crops. Lin et al. 2019). Zinc nanoparticles (ZnONPs) have been found to enhance the growth signs and antioxidant protection system activity of tomato plants (*Solanum lycopersicum*) under tomato mosaic virus (ToMV) stress in Egypt. The use of ZnONPs led to a significant increase in growth indices and photosynthetic attributes, as well as enzymatic and non-enzymatic antioxidants, while reducing oxidative injury caused by ToMV. ZnO-NPs could serve as a safe and cost-effective antiviral agent against ToMV in tomato crops. (Sofy et al., 2021). Different techniques were used to characterise the composition, size, and shape of the synthesised nanoparticles (NPs) and graphene. These techniques included FESEM, EDX, XRD, and FTIR spectroscopy and were used to analyse surface morphology, elemental analysis, Inorganic nanoparticles, such as calcium phosphate, iron, graphene, zinc, and silver nanoparticles, are highly stable, non-toxic, hydrophilic, and biocompatible materials that can be used for disease management. (Kurtjak et al., 2017). Inorganic NPs have antimicrobial properties due to their ions, which can generate reactive oxygen species (ROS) to eliminate pathogens. Zinc and copper ions are effective at killing pathogens by inducing ROS generation. Al and silver nanoparticles are also toxic to bacteria at low concentrations and disrupt pathogen cell membranes. Nanoparticles can also intercalate into DNA, and metal ions can bind to cellular proteins, leading to protein deactivation and precipitation, which increases cellular concentrations and eventually results in cell death. (Mittapally et al., 2018). Polymeric nanoparticles (PNPs) are composed of biodegradable and nontoxic natural or synthetic polymers and are used for producing nanocapsules or nanospheres. (Stanisic et al.,2018). Nanotechnologies have mainly been used in medical research and animal science, but they can also be applied to agriculture to increase crop yield and improve plant protection. Nanoparticles have the ability to deliver genes into plant cells, leading to diseaseresistant plants and improved crop species. This technology uses small particles made of metals or polymers to protect and transport genetic material. Despite some challenges, such as potential toxicity and unintended genetic changes, nanoparticle-mediated gene delivery offers a promising approach for sustainable and efficient crop improvement. This technology can also help in investigating plant genomics and gene functions, making plants resistant to pathogens and pests, and improving crop quality. Engineered nanoparticles with nucleotides, such as siRNAs, can be used for smart delivery and engineering disease resistance in crops. (Hamid, A., and Saleem, S., 2022).

2.Materials and method

2.1. Green synthesis of graphene nanoparticles from leaf extract and characterization

The green synthesis of nanoparticles is an emerging field of research that focuses on developing eco-friendly and sustainable methods for producing nanomaterials using natural resources. One such approach is the use of plant extracts as reducing agents and stabilisers for nanoparticle synthesis. The synthesis of graphene nanoparticles using leaf extracts has gained significant attention due to its potential applications in a variety of fields, such as biomedicine, energy storage, and catalysis. Characterization of these nanoparticles is essential for understanding their properties and performance, and various analytical techniques such as TEM, X-ray diffraction, ultravioletvisible spectroscopy, and Fourier transform infrared spectroscopy are commonly used for this purpose.
(Gupta, V. K., & Saleh, T. A. 2013).

2.2. Effect of graphene nanoparticles on tomato seed germination

To conduct a germination study, seeds with similar dimensions were chosen and sterilised with NaOCl before being rinsed thoroughly with Millipore water to remove any remaining NaOCl residue. The seeds were then soaked in graphene nanoparticle suspensions at different
concentrations (control, 30, 60, 90, and 120 mg L^{-1}) for two hours. The graphene nanoparticle treatment suspensions were prepared by sonication of the graphene stock suspension (100 W, 25 kHz) to ensure uniformity. Afterward, the seeds were soaked in graphene suspensions, placed onto qualitative paper in Petri dishes, and incubated for three days in a growth chamber under controlled environmental conditions. After that, the germinated seeds were transferred to cocopeat plastic seedling trays, and the germination rate and growth were analysed. (Xing et al., 2007).

2.3.Impact of graphene nanoparticles on tomato plant growth and parameters

Graphene nanoparticles are a relatively new material, and research on their effects on tomato seed germination is limited. Based on the research available on similar nanomaterials and their effects on plant growth and development, it is possible to make some general predictions about the potential effects of graphene nanoparticles on tomato seed germination. Firstly, it is important to note that the size, concentration, and surface properties of the nanoparticles can all affect their interactions with plants. Generally, smaller nanoparticles are more likely to be taken up by plants, and higher concentrations can have negative effects on growth and development. Certain types of nanoparticles can enhance or inhibit germination, depending on their concentration and properties. For example, silver nanoparticles have been shown to enhance seed germination in some plant species while inhibiting it in others. (Khot, L. R., and Sankaran 2012). Carbon nanotubes, on the other hand, have been found to inhibit seed germination and reduce plant In terms of the specific effects on tomato seed germination, research has shown that exposure to growth It is possible that exposure to graphene nanoparticles could have a negative effect on tomato seed germination if the nanoparticles are present at high concentrations or if they have certain properties that inhibit germination. The effect of graphene nanoparticles on tomato seed germination is currently uncertain and would require specific experimental data to determine. The impact of graphene nanoparticles on tomato plant growth and parameters is an area of active research, and the results are still inconclusive. Based on the available literature, it is possible to make some general predictions about the potential effects of graphene nanoparticles on tomato plant growth and parameters. Graphene nanoparticles are a relatively new nanomaterial, and their interactions with plants are not yet well understood. Exposure to carbon nanotubes has been found to reduce plant growth, inhibit photosynthesis, and alter the morphology of plant roots. (Khodakovskaya and M. de Silva, 2012). It is worth mentioning that the impact of nanoparticles on plant growth and characteristics can differ based on various factors such as the size, concentration, and surface characteristics of the nanoparticles, as well as the type of plant and its growing conditions. Certain research has indicated that exposure to nanoparticles may actually have beneficial effects on the growth and progress of plants. such as enhancing photosynthesis, improving nutrient uptake, and increasing resistance to pests and diseases.

2.4.Impact of graphene nanoparticles on tomato plant chlorophyll and protein content

The analysis of chlorophyll content in the seedling's leaves (measured as fresh mass) was conducted according to the method described by Arnon (1949). After treatment with graphene nanoparticles, the collected fresh leaves were crushed using chilled acetone and left to incubate overnight. The concentration was determined using the equations.(1), (2), and (3) given below:

Chlorophyll a (mg L^{-1}) = 12.72 (A663)– 2.59 (A*645) (1)

Chlorophyll b (mg L^{-1}) = 22.88 (A645)– 4.67 (A*663) (2)

Total Chlorophyll content (mg $L^{-}1$) = Chl a + Chl b (3)

To determine the protein concentration, the method described by (Liang et al. 2008) was followed, with bovine serum albumin as the standard. The spectrophotometer was used to measure both the chlorophyll and protein content. (Liang, W. & Zhou,Y. 2008). There is limited research on the impact of graphene nanoparticles, specifically on tomato plant chlorophyll and protein content. Chlorophyll is an important pigment that is essential for photosynthesis, which is the process by which plants produce energy from sunlight. Exposure to certain types of nanoparticles has been found to affect chlorophyll content in plants. For example, exposure to silver nanoparticles has been shown to decrease chlorophyll content in some plant species, while exposure to zinc nanoparticles has been found to increase it. Proteins are essential building blocks for plant growth and development, and exposure to nanoparticles can affect protein content in plants, while exposure to silver nanoparticles has been shown to decrease it. Regarding the specific effects of graphene nanoparticles on tomato plant chlorophyll and protein content, one study reported that exposure to graphene nanoparticles at low concentrations had significant positive effects on chlorophyll or protein content in tomato plants. The effects of graphene nanoparticles on tomato plant chlorophyll and protein content require further research to determine their specific impacts. There is limited research on the impact of graphene nanoparticles on tomato plant chlorophyll and protein content. However, based on research on similar nanomaterials and their effects on plant physiology (Wang C., Wang Z., et al.),

3.Determination and deactivation of TMV virus effects in plants

The experiment was conducted in a greenhouse using healthy tomato seeds (*Solanum lycopersicum*) to investigate whether Graphene could induce systemic resistance to TMV in tomato plants. The greenhouse was maintained at a temperature of 25 °C, and seeds grow in trays, seeds filled with sterilised sandy loam soil. After waiting for seven days, seedlings that were the same size were picked and moved to 15-cm pots filled with 10 kg of soil. The pots were put in insect-proof cages inside the greenhouse.

3.1. The Foliar Treatments of Graphene NPs

The TMV virus was inoculated into the tomato seedlings. The seedlings were at the 4-8 leaf stage and were equally treated with foliar applications of graphene NPs at concentrations of 30, 60, 90, and 120 mg/L. The foliar applications were sprayed onto all plants except control and were applied until runoff was achieved.

4.Source of viruses

A leaf that had been infected with TMV was taken from a tomato farm. The presence of TMV was confirmed by observing the characteristic symptoms of a viral infection.

4.2. Evidence of infection

After Twenty days plants inoculated with TMV, the degree of viral infection was assessed and calculated in comparison to the infected control. and also evaluated by examining all plants in each treatment. The symptoms were recorded using a rating scale for vein clearing in leaves showing mosaic symptoms.

Treatment	Graphene Np's concentration
Control	Distilled water
Sample 1	30ppm
Sample 2	60 ppm
Sample 3	90 ppm
Sample 4	120 ppm

Table 1 Foliar application and graphene nanoparticles treatments with incresing concentration under the greenhouse conditions.

5.Infected plant photosynthetic pigments determination

The method outlined by Lichtenthaler and Buschmann (2001) was used to estimate the concentration of both chlorophyll a and b in fresh leaves. This involved employing a specific technique to accurately determine the quantity of each type of chlorophyll present in the leaves that had not undergone any processing.

6.Results and discussion

In this study, the experiment performed on three main topics: first, the synthesis of graphene nanoparticles; second, the nanoparticles effect on seed germination; and third, the deactivation of TMV viral effects or symptoms on *Solanum*

4.1.Experimental design

The experiment involved two different conditions, first the inoculation of tomato plants with TMV, and second, the treatment of viral effect using the plants with graphene nanoparticles. The tomato plants used in the experiment were 21 days old and were divided into 2 treatments, with each treatment consisting of one pot containing two plants. The plants were maintained under controlled conditions and were arranged in a completely blocked design. The temperature was maintained between 22-28 ºC, and the plants were watered as needed. To inoculate the plants with TMV, healthy tomato plants were mechanically infected with a virus using a phosphate buffer solution. The symptoms of the virus were noted after 20 days.

lycopersicum and the main focus on the second and third experiment.

7.Synthesis of graphene nanoparticles and characterization

Collect fresh leaves from a nursery and wash them thoroughly with distilled water to remove any impurities. Grind the leaves in a blender and pestle to obtain a fine paste. Add the paste to a beaker containing deionized water or a suitable solvent, and stir the mixture for 1-2 hours to extract the active compounds from the leaves. Filter the resulting extract through a filter paper to obtain a clear solution. Prepare a solution of graphene precursor by dissolving it in deionized water or a suitable solvent. Add the leaf extract solution to the graphene precursor solution and stir the mixture vigorously for several hours at room temperature to allow for the reduction of graphene and the formation of graphene nanoparticles. Heat the mixture to a suitable temperature, typically between 60 and 80°C, and maintain this temperature for a few hours to allow for the complete reduction of the graphene and the formation of graphene nanoparticles. After the reaction is complete, filter the solution through a filter paper to remove any impurities, and wash the resulting graphene nanoparticles with distilled water to remove any residual reactants or byproducts. Dry the graphene nanoparticles using a suitable method, such as air-drying.Characterise the graphene nanoparticles using suitable analytical techniques, such as UV-Vis spectroscopy, which is a technique used to measure the absorbance of light by nanoparticles. This technique can be used to determine the size, shape, and concentration of nanoparticles in a sample. It's important to note that the exact protocol and procedure may vary depending on the specific leaf extract and graphene precursor used, as well as the desired properties of the graphene nanoparticles. It is also important to use it safely and conduct experiments.

7.1Impact of graphene nanoparticles on tomato plant seeds germination

Tomato seeds of approximately equal dimensions were chosen and subjected to surface sterilisation with NaOCl. After washing with Millipore water to remove any residual NaOCl, the seeds were treated with different concentrations (30 ,60 ,90 , and 120 mg/L) of graphene suspensions for 2 hours. The suspensions were prepared by synthesising a stock solution and subjecting it to ultrasonic vibration. 4 seeds and 1 control seed were then placed on a Petri dish containing qualitative filter paper and incubated in a growth chamber with controlled environmental conditions for 3 days. Then seed was transferred into a coco peat plastic seedling tray and provided at optimum temperature. in green house condition after 21day The germination of the seeds was recorded and analysed. Graphene nanoparticles may enhance the physiological processes involved in seed germination and early growth.

7. 2.Impact on growth parameters The exposure of tomato seedlings to graphene resulted in a significant increase in both root and shoot length, with the highest values observed in plants treated with 120 mg L^{-1} of graphene nanoparticles. In contrast, the lowest root and shoot heights were observed in plants treated with 30 mg L^{-1} of graphene. The mechanism behind graphene's phytotoxicity is not well understood. The effects of graphene concentrations on the fresh and dry weight of roots and shoots were also analysed, and significant variations were observed among control and graphene-treated plants. Both the root-shoot fresh and dry weight increased with higher graphene treatments. The highest fresh and dry weights of roots and shoots were obtained at a concentration of 120 mg L-1 of graphene after 21 days of treatment. This increase in fresh and dry weight may be attributed to the increase in growth parameters such as root-shoot length with increasing graphene concentrations**.**we can seen in figure (1) The positive effect of Graphene nanoparticles tomato seedling plants

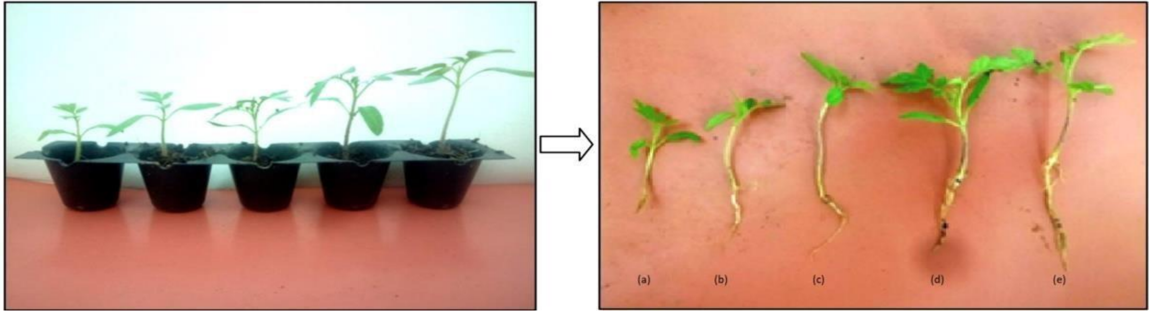

Fig. 1 Graphene Np's effect concentration wise on *Solanum lycopersicum* plant root shoot development. (a) Control (b) 30ppm graphene nanoparticles treatment showing small growth of root and shoot (c) 60ppm nanoparticles treatment (d) 90ppm nano particles treatment (e) 120ppm graphene nanoparticles treatment showing large growth of root and shoot.

Graphene Np`s conc.($mg.l^{-1}$)	Average Root lenth (cm)	Average Shoot length(cm)	Average Frash weight (gm)	Average Dry weight(gm)
0.0	1.10 cm	3.29 cm	1.53	0.32
30	1.60 cm	3.62 cm	1.80	0.50
60	2.08 cm	3.90 cm	2.06	0.71
90	2.40 cm	4.20 cm	2.79	0.92
120	2.50 cm	4.25 cm	2.94	0.96

Table 2 Shows the average number of root shoot lengths, dry weights, and fresh weights after treating the seedling with graphene NPs at concentrations, 0.0, 30, 60, 90, and 120. ($mg.l^{-1}$).

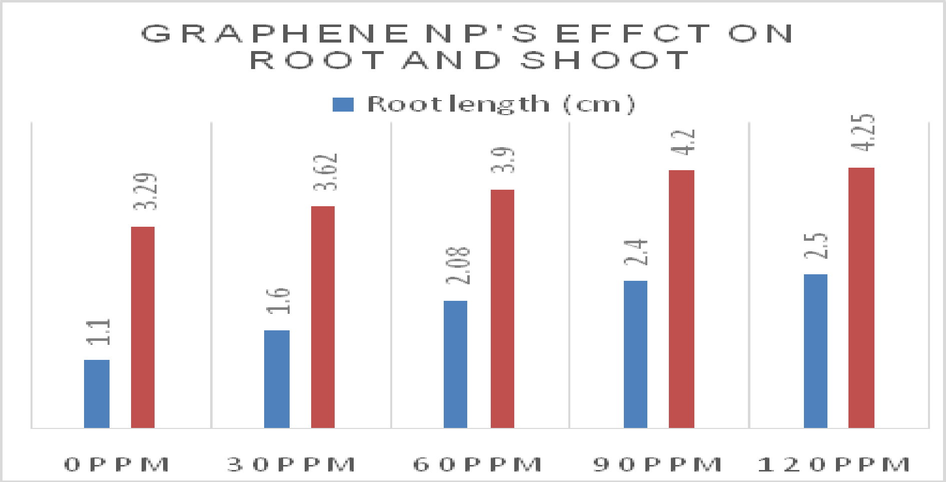

Fig.2 Root and shoot lengths, after treating the seedling with graphene NPs at concentrations, 0.0, 30, 60, 90, and 120. (mg.l^{-1}).

7.3. Graphene nanoparticles concentrations Impact on chl a & chl b and protein content

Table 3 displays the impact of graphene on the levels of chl. Content including Chlorophyll a, Chlorophyll b, and total Chlorophyll . It is evident that graphene concentrations have a significant effect on the chlorophyll content of tomato seedlings. The maximum amounts of chl. a, b, and total content were observed to be 2.34, 1.68, and 4.02 mg g^{-1} fresh wt, respectively The function of chlorophyll-a is integral to the proper functioning of both the light-harvesting complex and PSII

Plant	**Treatment** (Mg. L^{-1})	**Photo pigment concentrations**		**Total** Chl. (mg.g^{-1}f.wt)
		Chlorophyll a (mg.g^{-1}f.wt).	Chlorophyll b(mg.g^{1}f.wt)	
Control	0.0	1.01	0.23	1.24
Sample 1	30	1.36	0.38	1.74
Sample 2	60	1.70	0.70	2.40
Sample 3	90	1.93	1.01	2.94
Sample 4	120	2.34	1.68	4.02

Table 3: Photo pigment concentrations after treatment with graphene nanoparticles (mg.g^{-1}f.wt).

reaction centre. Specifically chlorophyll-a functions as an electron donor in the photosynthetic electron transport chain. Its role is crucial for facilitating the transfer of electrons within this chain and for the overall process of photosynthesis to occur.

8.Pot experiment.

The experiment was conducted in two stages. In the first stage, fresh tomato plants were inoculated with TMV, and in the second stage, graphene nanoparticles were applied to deactivate the virus's effects and symptoms**.**

8.1.Virus Infectivity

curling and yellow spots (as shown in Figure 2). The infected tomato plants were foliar-applied with graphene NPs at concentrations of 30, 60, 90, and 120 mg/l. Two treatments of graphene nanoparticles were applied, with the first treatment administered 20 days after infection and second treatment applied next ten days.

8.2. Reduction of virus infectivity

Figure 4 Show the current sudy was carried out after

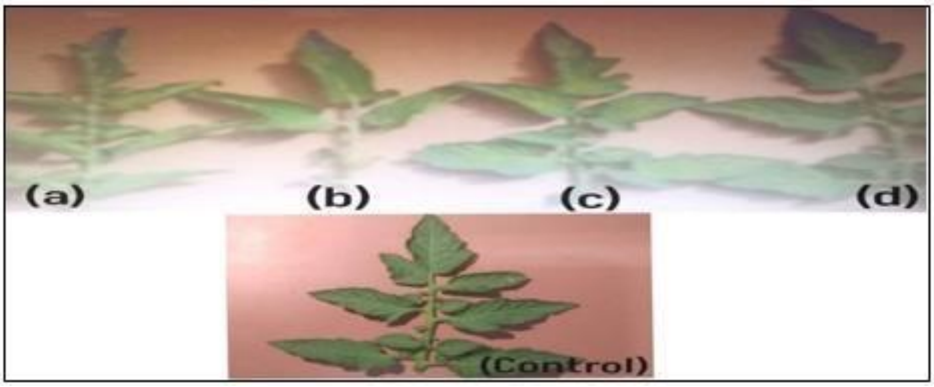

Fig. 4. Effect of treatment with Graphene NPs on *Solanum lycopersicum* plants compared with healthy controls Healthy control. tomato plants (a) Tomato plants infected with TMV are treated only with 30 ppm Graphene NPs. (b) Tomato plants treated with 60 ppm graphene NPs (c) A tomato plant treated with 90 ppm graphene showed fewer symptoms. (d) A tomato plant treated with 120 ppm graphene NPs showed no symptoms in the plant.

Fig.3 TMV symptom appearance after 20 days of inoculation with the TMV virus.

Figure.3 show mosaic symptom after inoculation of viral infection.in this experiment The pots are used in diameter of 15 cm and a depth of 20 cm, and each pot contained 2 kg of soil and two plants. The plants were maintained under controlled conditions with temperatures ranging from 22-28°C and were watered as required. Twenty-oneday-old tomato plants were infected with TMV using a phosphate buffer solution through mechanical inoculation. The symptoms of the virus were recorded after 20 days of inoculation, with each plant exhibiting symptoms such as leaf

systemic symptoms appeared 20 days after inoculation with the TMV virus. The effectiveness of graphene nanoparticles in suppressing plant virus infection in tomato plants was evaluated by verifying the presence of TMV through the observation of distinct viral symptoms on various host plants. In these studies, foliar application of 30 ppm graphene NPs reduces small quantities of TMV viral symptoms, but 120 ppm of graphene nanoparticles reduces 95% of TMV symptoms and infections. Phenotypically identify viral infection, and tomato plants showed reductions of virus infection. In concentration, 30 plant leaves show more number spots, and curling is very high. and in the 60 ppm concentration of graphene NPs, how much less spots and infection compare than 30 ppm in the third concentration of 90 ppm, showing the less number of spots and curling of the leaf. and last 120 ppm approximate all reduce. The infection

8.3.Relative concentration of TMV

Table 4 It can be inferred that foliar application of graphene nanoparticles was carried out on 21dayold TMV-infected plants, with the first treatment applied 10 days after infection and the 2nd treatment applied ten days later. The optical density of the DAS ELISA at 405 nm was measured to determine the relative concentration of TMV in the plants. The results showed that treatment with caused by the virus was reduced, indicating that graphene nanoparticles can act as efficient antiviral agents. According to several studies, nanoparticles can attach themselves to virus particles and impede virus replication within host plants. In this case, the graphene nanoparticles attached to the protein virus particles. (Jain and Kothari, 2014)

graphene NPs at a concentration of 30 ppm resulted in the lowest relative concentration of TMV, followed by 60 ppm, 90 ppm, and 120 ppm. In other words, increasing the concentration of graphene NPs led to a decrease in the viral appearance in the plant, indicating that graphene NPs had a dose-dependent effect on deactivating TMV.

Treatments (ppm)	**O.D of DAS ELISA (405 nm)**
	TMV
Control	0.00
30 ppm	0.304
60 ppm	0.263
90 ppm	0.165
120 ppm	0.101

Table 4: Effect of graphene NP concentrations on TMV, DAS ELISA was employed to determine the relative concentrations of the virus in tomato plants.

DAS-ELISA is a widely used technique for the detection of plant viruses, including *Tobacco mosaic virus* (TMV). The basic principle of DASELISA is to capture the virus with an antibody that is specific to the virus, and then detect it using a secondary antibody conjugated with an enzyme. In the case of TMV, the viral protein coat is usually used as the target for the capture antibody. The 405 nm wavelength is commonly used as the detection wavelength in ELISA because it is in the visible range of the electromagnetic spectrum and can be easily detected by most ELISA readers. However, the specific wavelength used may vary depending on the type of enzyme substrate used in the assay. The DAS-ELISA protocol for TMV detection typically involves the following steps: Preparation of plant samples: Leaf tissues from the plants to be tested are ground in a buffer solution to extract the viral particles. Coating the ELISA plate: The wells of an ELISA plate are coated with the capture antibody specific to the TMV protein coat. Incubation: The plant extract is added to the wells and incubated, allowing the TMV particles to bind to the capture antibody. Washing: The wells are washed to remove any unbound plant material. Detection Following this, a secondary antibody that is attached to an enzyme (such as horseradish peroxidase) is introduced into the wells. which binds to the captured virus particles. Colour development: A substrate solution is added to the wells, which reacts with the enzyme to produce a coloured product. Reading the absorbance: The absorbance of the coloured product is read using an ELISA reader set to a 405 nm wavelength. The higher the absorbance, the more TMV particles were present in the original plant extract. DAS-ELISA is a highly sensitive and specific technique for the detection of TMV and other plant viruses. It is a useful tool for plant pathologists, virologists, and plant breeders in their efforts to manage viral diseases in plants. The results suggest that graphene nanoparticles may be effective in deactivating TMV by interfering with RNA copying during viral multiplication. Previous studies have reported the antiviral activity of graphene-based nanomaterials, such as graphene nanoparticles, by binding to viral nucleic acids and inhibiting their replication. Smaller graphene nanoparticles can even penetrate the viral structure and directly affect viral replication. Additionally, graphene nanoparticle application can increase the content of certain compounds, indicating an adaptive response to the viral infection likely due to the antioxidant properties of graphene nanoparticles, which reduce oxidative stress and improve overall plant health.

8.4. Infected plant photosynthetic pigments determination

Table 5 Indicates that there were significant differences in the levels of all the investigated photosynthetic pigments chl. a and chl. b between the infected and healthy plants The increase in the levels of the aforementioned pigments in infected tomato plants is thought to be caused by a reduction in reactive oxygen species (ROS), which are mainly located in the chloroplast membranes in leaf tissues. With regard to the influence of graphene nanoparticles ,all the plants treated with varying concentrations of graphene NPs showed a noteworthy rise in comparison to the healthy control. Additionally, treatment with graphene NPs at 120 ppm resulted in the enhancement of all studied photosynthetic pigments when compared to the infected control. Notably, the highest concentrations of graphene NPs at 90 and 120 ppm demonstrated a significant increase in the levels of the pigments compared to the other treatments, indicating a positive impact of graphene NPs on these photosynthetic pigments overall.

Treatments	TMV		
	Chlorophylls		Total chl. a & chl. b
	Chl. a	Chl. b	
Healthy Control	2.76	1.47	4.23
ppm	2.85	1.61	4.46
ppm	3.15	1.85	5.00
ppm	3.25	1.95	5.20
ppm	3.38	2.12	5.50

Table.5 TMV infected plant after curing measuring the photosynthetic pigment The concentrations in (mg.g1f.wt).

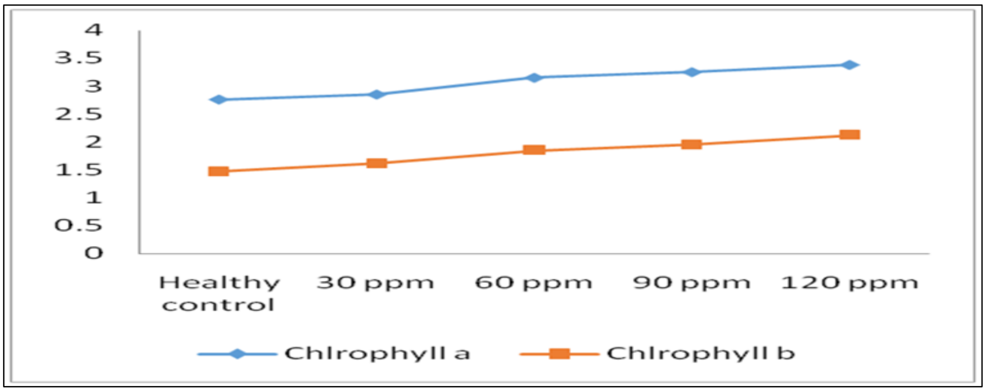

Fig.5 TMV infected plant after curing measuring the photosynthetic pigment The concentrations in (mg.g1f.wt).

9.Discussion

Graphene nanoparticles were synthesised in the study using leaf extract. Graphene nanoparticles enhance the seed germination rate and root shoot development. Graphene nanomaterials nanomaterial provides a promising approach for controlling the spread of the *Tobacco mosaic virus* in *Solanum lycopersicum* plants. The effectiveness of the treatment was evaluated by observing the symptoms of the tomato plants and comparing the results with those of the control group. The results showed that the use of the nanoparticles significantly reduced the symptoms of the virus and improved the growth of the plants. The mechanism by which the nanoparticles deactivate the virus is still unclear and requires further investigation. However. The confirmation of TMV infection was based on the identification of

characteristic viral symptoms that manifested on various host plants, with systemic symptoms typically appearing 20 days post-inoculation. Notably, foliar application of 120 ppm of graphene nanoparticles on tomato plants resulted in a reduction in virus infection, indicating the potential of graphene nanoparticles as effective antiviral agents. The study's findings also suggest that graphene nanoparticles bind to virus particles and neutralize their effects in host plants.

10.Research objectives

The primary objectives of this research are as Synthesise nanomaterials, graphene nanoparticles, Characterise the synthesised nanomaterials using techniques, including UV-Vis spectroscopy,.Evaluate the antiviral activity of the synthesised nanomaterials against TMV in *Solanum lycopersicum* plants under controlled conditions. Study the mechanism of action of the nanomaterials in deactivating TMV in tomato plants. Assess the phytotoxicity of the nanomaterials on tomato plants and their impact on plant growth and development.

11.Conclusion

This research will provide important insights into the synthesis of graphene nanomaterials by using leaf extracts, and these nanomaterials enhance root shoot development in *Solanum lycopersicum* plants. And the potential use of nanomaterials for the management of TMV infection in *Solanum lycopersicum* plants. and study about the proteins and chlorophyll pigment. The outcomes of this research may contribute to the development of sustainable and eco-friendly approaches for controlling plant diseases, which can have significant economic and environmental benefits. The findings of this research may also have implications for the use of nanomaterials in other areas of agriculture, such as the management of other plant diseases and pests.

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13.Ethical approval

I confirm that the authors did not conduct any experiments involving humans or animals.

14.Declaration of competing interest

I confirm that the authors have declared that they do not have any conflicts of interest.

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