Egypt. J. Chem. **Vol. 65**, No. 9 pp 171 - 187 (2022)

Egyptian Journal of Chemistry

http://ejchem.journals.ekb.eg/

Impact of engineered nano silver on root- knot nematode, *Meloidogyne incognita* and on DNA damage in tomato plants under screen house conditions

Shoaib R. M.,[1] A. B. Abdel-Razik,[2] Naglaa, M. Ebeed[2] R. M. Esmail,[1] and Entsar, H. Taha[3]*

[1]*Genetic and Cytology Dept., Genetic Engineering and Biotechnology Division, National Research Centre, Dokki, Cairo, Egypt.*

[2]*Department of Genetics, Faculty of Agriculture, Ain Shams University*

[3]*Department of Plant Protection, Faculty of Agriculture, Ain Shams University*

Abstract

Root-knot nematodes are one of serious pathogens which cause economically losses to their host plants. Nanotechnology is a fast-growing technology in nematode and other pathogens management to avoid the hazard effects of chemical pesticides and nematicides on human health, non-target organisms and ecosystems. The effects of Ag engineered nanoparticles (AgENPs) synthesized by green method using plants with nematicidal properties or reductive potential such as *Curcuma comosa* (N1), *Cycas circinalis* (N2), Chitosan (N3) and *Crotalaria juncea* (N4) were tested. This study was done to investigate the genotoxic evaluation of the four AgENPs and the analysis of its effects through the assessment of pathogenicity and reproductivity of *Meloidogyne incognita,* plant growth parameters, silver residue, activity of antioxidative enzymes and assessment of DNA damage. The experiment was conducted using three old tomato seedlings which transplanted, treated with three concentrations of four AgENPs and infected with 1000 IJs *M. incognita* under screen house condition. The data demonstrated that, all nematode parameters were suppressed using the different concentrations of AgENPs. The suppressive effect was increased proportionally with the Ag concentrations. Moreover, the damage in DNA parameters was increased proportionally with concentration of AgENPs. Silver residue in tomato fruits after application is relatively very low.

Keywords: AgENPs, genotoxicity**,** root-knot nematodes, tomato plants, nano residue

Introduction

Progression in nanotechnology and its impacts have brought up concerns about the application of engineered nanoparticles (ENPs) in various sectors of the economy, including the field of agronomy[1]**.** Silver is widely utilized in this technology because of its activity as biocides[2] which due to its unique physical and chemical structures[3]. Root-knot nematodes (*Meloidogyne* spp.) are considered the most serious plant parasitic nematode which attach several plant hosts and can cause about 90% of crops loss[4]. New approaches such as nanoparticles (NPs) are promising in plant pathogen control to avoid chemical hazards[5]**,** such as fungal pathogens[6], bacteria[7]**,** and nematodes[2,8,9]**.**

The genotoxicity of chemicals is the most important adverse effect on human, non-target organisms and environmental safety[10]**.** A few reports are indicative on DNA damage and genotoxicity in plants[11]**.** In particular, the behavior of NPs in natural conditions and their consequent ecological effects are still poorly understood. Clearly, the safety of NPs must be established when considering further the development of further applications for nanotechnology[12]**.** Nanoparticles are often synthesized using different chemical techniques such as chemical reduction, solvo-thermal reduction, electrochemical techniques, and photochemical reaction. Using of a chemical reducing agent consumes more energy and generates larger sized particles in addition to the toxicity of some chemical reductive which make it non-eco-friendly in nature[13]. Additionally, the chemically synthesized NPs were found to show less stability[14]. Hence, alternate ecofriendly protocols should be adopted which can utilize bacteria, fungi and plant extracts or natural products as reducing agents which are efficient reductives and produce more stable and dispersible NPs, more small particles, consuming less energy[15]. These biological/green synthesis methods are not only environmentally friendly but also low cost-effective, more rapid, and more proficient than the chemical procedures[14].

*Corresponding author e-mail: dr.entsar_elsayed@agr.asu.edu.eg

Received date 21 September 2021; revised date 27 September 2021; accepted date 03 October 2021

DOI: 10.21608/EJCHEM.2021.97189.4547

Using some nematicidal plant materials such as *Curcuma comosa* (N1), *Cycas circinalis* (N2), *Chitosan* (N3) and *Crotalaria juncea* (N4), in the silver green synthesis may lead to avoid the toxicity of chemical reductives and decreasing the AgNPs concentrations which need to manage the plant parasitic nematodes, for example, Chitosan is a form of Chitin which has a nematicidal effect on root-knot nematodes and other plant parasitic nematodes without any toxicity on human, animals, and plants[16,17]. *Crotalaria* has approved a suppressive effect on root-knot nematodes[8,18] pyrrolizidine alkaloids and monocrotaline are the toxic compound in this plant[19]. *Curcuma longa* may use as a biological reductive agent which improve the nematicidal effect of nano due to their enrichment of some alkaloids and flavonoids. In contrast, Cycas has no effect on nematode itself, but it can improve the efficiency of the nanoparticles itself [3] by increasing the reduction process and the stability of nano particles[3,20].

Over the past decade, the single-cell gel electrophoresis, or comet assay, has become one of the standard methods for assessing DNA damage and has been used to find the genotoxicity of nanoparticles due to its reliability, simplicity, low cost, sensitivity, and versatility[21,22]**.** Biomarkers are altered biochemical or physiological responses, or morphological parameters caused by exposure of an organism to a certain potentially toxic substance that can be measured in its cells, body fluids, tissues, or organs[23,24]. Biomarker analyses have gained prominence as they offer the possibility of early contamination detection by toxic substances, as well as a more realistic assessment of the effects of this contamination[25]. Many NPs have been tested in terms of their uptake, translocation, accumulation and phytotoxicity, and the effects on morphological, anatomical, physiological, biochemical and genetic characteristics of certain plants were studied. NPs cause positive as well as negative changes in exposed plants[26].

Thus, this study was done to investigate the toxicological evaluation of the four green AgENPs synthesis and the analysis of its genotoxic effects through the assessment of pathogenicity and reproductivity of *M. incognita,* plant growth parameters, Ag accumulation in tomatoes fruit, antioxidative enzymes activity and DNA damage by comet assay in tomato plant roots.

MATERIALS AND METHODS

Study area: The study was carried out at the Department of Plant Protection, Faculty of Agriculture, Ain Shams University

Root-knot nematode culture:

The culture of *Meloidogyne incognita* (Mi) was reared in the screen-house on eggplant (*Solanum melongena*), in sterile sandy clay soil. The second infective juveniles (2IJs) were extracted from galled roots by washing the infected roots and cutting into small pieces, and then placed in the mist chamber for egg hatching. The 2IJs were collected and refrigerated for the experimental use.

Nano materials

Nano materials were obtained from Nano fab, Egypt, which prepared as following

Synthesis of silver nanoparticles

The silver nitrate ($AgNO_3$) used in this experiment was obtained from Sigma Aldrich. 3 ml of Plant extract was added to 60 ml of 10^{-3} M $AgNO_3$ solution, and the reaction was left to take place at ambient conditions. The observed change in color from colorless to transparent yellow and finally to a dark brown with time indicates the formation of AgNPs. Reduction of the Ag^+ ions was monitored with respect to time using UV–visible spectral analysis. Once the reaction mixture reached a dark brown color, it was then centrifuged to collect the AgNPs. The nanoparticles have been washed three times using deionized water and were then re-suspended in 95 % ethanol (Fisher Scientific) prior to characterization. The pH value of the green synthesis of nanoparticles has been maintained at 9. The nanoparticles were kept in sonicator for 10 min

Preparation of Colloidal Curcumin, Cycas and Crotalaria Nanoparticles

The extract was made using 20 g of fresh leaves of *Curcumin*, *Cycas* and *Crotalaria*. Prior to extract preparation, the plant leaves were cleaned thoroughly using deionized water and then cut into small pieces. The leaves were then added into 125 ml of boiling deionized water, and left to boil for 3 min. The solution was then removed from the heat source and left to cool to ambient temperature (approximately 25° C). The extract was then filtered through a coarse sieve to remove any leaf matter and the resultant filtrate was then refrigerated. The Plant extract was collected and stored at 4° C. Finally, the extract was used for the synthesis of AgENPs. nanosuspension were prepared by solvent-antisolvent precipitation method. Briefly, they were extracted in ethanolic solution with concentration 1.3 % wt/vol under vigorous stirring at 60 C overnight. Then, the mixture was filtered using Whatman filter paper. The extracted filtrate was then subjected to vigorous stirring at heat at 60 C. Then about 30% vol/vol H_2O has been added to ethanolic solution as an anti-solvent and PVP 10 %wt/vol added under vigorous stirring and heat at 60 C for another 3 hr. A milky yellowish nano-colloidal solution has been obtained indicating to formation of *Curcumin*, *Cycas* and *Crotalaria* nano-colloids.

Preparation of Colloidal Chitosan Nanoparticles

Chitosan nanoparticles were prepared via ion gelation method. Briefly, an appropriate concentration of chitosan dissolved into 1%v/v aqueous soln. of acetic acid under vigorous stirring at 60° C overnight till get a clear solution. Then an

aqueous soln. of cross linker agent TPP was added dropwise to the CS solution under vigorous stirring, where the ratio of CS to TPP was about 5 to 1. The reaction was left under vigorous stirring for 30 min after addition. Then the solution was washed and centrifuged at 8000 rpm for 20 min several time till the pH will be at 7. The chitosan nanoparticles were subjected to Freeze Dryer to obtain the nano powder and redispersed into distilled water.

Nanoparticles characterization

The color change of reaction mixture (silver nitrate solution and leaf extract) with respect to time is observed. The color changes at 15 min from colorless to faint yellow, indicating the formation of AgNPs. As time elapsed, the yellow-colored solution eventually became dark brown at 120 min, which is due to the increasing concentration of AgNPs as well as the growth of the particles in size. There is no significant change beyond 180 min, indicating the completion of the reduction reaction. This was further confirmed by UV–Vis spectroscopic analysis.

Transmission Electron Microscopy (TEM)

Shape and size of AgENPs were practically obtained using TEM. Specimens for TEM measurements were prepared by placing a drop of colloidal solution on 400 mesh copper grid coated by an amorphous carbon film and evaporating the solvent in air at room temperature. The average diameter of the prepared AgNPs was determined from the diameter of 2-100 nanoparticles found in several arbitrarily chosen areas in enlarged microphotographs.

Screenhouse experiments

Screenhouse experiments were carried out using three-week-old seedlings of tomato (*Lycopersicon esculentum* L. Castel Rock) 20, 40 ppm). Each of AgENPs concentration was repeated 20 times and subdivided into four groups each one was inoculated with one of the tested nematode concentrations: 0, 500, 1000, and 2000 IJs at the same time of nano addition. All the previous treatments were replicated for each AgENPs, another group of replicates that did not receive AgENPs were used as a positive check which were inoculated with the same concentrations of nematodes inoculum. The negative check treatments were represented by untreated neither AgENPs nor nematode. The experiment was ended after 60 days. The following data were recorded and calculated: nematode parameters, plant growth parameters, antioxidant enzymes activity, photosynthetic pigments, Ag residue and DNA damage.

Effect of AgENPs on antioxidant enzymes activity

Extraction of antioxidant enzymes

The first and second young leaves of tomato were used for detection of SOD, CAT, POX and PPO antioxidant enzymes. In this regard, 2 g were homogenized with 10 ml of phosphate buffer pH 6.8 (0.1 M), then centrifuge at 2^{o}C for 20 min at 20000 rpm in a refrigerated centrifuge. The clear supernatant (containing the enzymes) was taken as the enzymes source[27].

Determination of antioxidant enzymes

SOD activity was determined by measuring the inhibition of the auto-oxidation of pyrogallol using a method described by **Marklund and Marklund[28]; Kong *et al.*[29].** CAT activity was assayed according to the method of **Chen *et al.*[30].** POX activity was assayed according to **Bergmeyer[31]; Kong *et al.*,[29]. PPO** activity was determined by using a method described by **Kar and Mishra[32]; Razinger *et al.*,[33]**.

Effect of AgENPs on photosynthetic pigments

The method was used for the quantitative determination of chlorophyll a, b **Vernon and Selly[34].** The optical density of the plant extract was measured using spectrophotometer of two wave lengths (649 and 665 nm). These are positions in the spectrum where maximum absorption by chlorophyll (a) and (b) occurs. The concentrations of chlorophyll (a), (b) and total chlorophyll in plant tissue were calculated using the equations mentioned by **Vernon and Selly[34]**. For carotenoids, the concentration was carried according to **Lichtentahler[35]** .

Effects of AgENPs on Ag residue in tomato fruits

Samples of tomato were dried at 70 C° in an oven until constant weight then pulverized to pass a 1 mm sieve. Dry samples of 0.5 g were taken and digested using the wet digestion method by using a mixture of sulphuric acid 98% and hydrogen per- oxide 30% as described by **Thomas *et al.*, [36]**.

Chemicals and Reagents:

Standards solution 1000 µg/ml of Ag traceable to **NIST[37]** were procured from Scharlau Chemie, Spain. Nitric acid and Hydrochloric acid AR grade were procured from Merck Specialist Chemical limited. All glassware used was “A” grade and calibrated. Calibrated micropipette with range 100µl to 1000µl was used. Whatman filter paper no. 41 was used for filtration.

Instrumentation:

A radial view Spectro Ciros CCD ICP-OES (Spectro Analytical Instruments, Kleve, Germany) was used with settings optimization. Robustness of the plasma was calculated using the Mg (II) (280.2 nm): Mg (I) (285.2 nm) ratio from a 20 mg Mg L_1 solution and increased from 6.00 at 1300 W to 8.39 at 1500 W, robustness was 7.94 which is considered acceptable

ICP-OES conditions

Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES) with radial torch equipped with argon saturation assembly was used for the determination of lead and cadmium. High purity (99.99%) argon was used as plasma, auxiliary and nebulizer gas. The gas flows were kept at 15.0 l/min for plasma, 1.50 l/min for auxiliary and 0.56 l/min for nebulizer. Radio frequency (R.F) power of the

plasma generator was 1.35 kW. Vertical height of the plasma was fixed at 7 mm. Sample uptake time of 30.0 sec, delay time of 5 sec, rinse time of 10 sec, initial stabilization time of 10 sec and time between replicate analyses of 5 sec was maintained throughout the studies for ICP-OES. The instrument was calibrated for various parameters before the studies.

ICP-OES model:

ICP-OES Model Spectro Ciros CCD radial view- Software SpectroView 3.30 build 1032- Power (W)- 1450 W- Flow rate (Ar) 13 L min_1- Auxiliary Flow (Ar) 1.5 L min_1 -Nebulizer flowa(Ar) 0.71 L min_1- Nebulizer Modified Lichte- Spray Chamber Double pass cyclonic - Torch injector diameter 1.8 mm - Sample uptake rate 2.0 mL min_1 -Sampling strategy Phase 1: 3 s Phase 2: 3 s- Phase 3: 3 s Phase 4: 6 s - Phase 5: 10 s

Effect of AgENPs on DNA damage

DNA Extraction

Individual tomato root sample was removed from the plant and placed in a petri dish with Sörensen buffer (dimethyl sulfoxide (DMSO), 0.1 mM ethylenediaminetetraacetic acid (EDTA), 50 mM sodium phosphate, pH 6.8, 0.5% kept on ice. The root tissue was gently sliced by a razor blade and the resulting material was repeatedly dipped in the cold Sörensen buffer. The suspension with released nuclei was filtered through a 30 µm disposable filter (Partec, Műnster, Germany) to remove most of the debris and centrifuged at 550 g for 5 min at 4ºC **Georgieva and Stoilov**[38].

Preparation of alkaline Comet assay

The protocol described by **Georgieva and Stoilov**[39] with modifications was followed. Microscope slides were coated with 0.5% normal melting agarose and dried at room temperature. Forty µl of the nuclei suspension was mixed with 40 µl of 0.1% low melting agarose, spread on the slide surface and subjected to gel formation for at least 10–15 min on a cooling plate at 4°C. Lysis was carried out in 2.5 M NaCl, 10 mM Na_2EDTA (pH8), 10 mM Tris-HCl (pH8), 1% N-lauroylsarcosine sodium salt, 1% TritonX-100, 10% DMSO for 15 min at 4°C in the dark. Electrophoresis was performed in prepared TAE buffer (pH8) at 0.5, 1, 2 and 5 V/cm for 10 min for root nuclei. The slides were dehydrated in 70% and 96% ethanol for 5 min and dried at room temperature. The slides were covered with solution of the fluorescent dye acridine orange (10µg/ml). Visualization of the stained comets was carried out using a fluorescence microscope (Zeiss Jenamed-2) coupled with a digital camera (Samsung Digimax V50). Three independent experiments were performed, and 50 comets were analyzed per point in each experiment. Damage was detected according to the fragments intensity which migrated during electrophoresis[42].

Statistical analysis

The data of all experiments were statistically analyzed using analysis of variance procedure proposed by **Snedecor and Cochran**[40]**.** The differences between means were compared using Duncan's Multiple Range Test **Duncan**[41].

RESULTS

Morphological Structure of AgENPs:

Fig. 1 shows the morphological properties of as-prepared AgNPs with a predominate shape of spheres in the presence of different herbal extracts including *curcumin*, *Cycas*, chitosan and *Crotalaria* respectively. It is clear that, the average particle for *curcumin* capped AgNPs was about 15 ± 5 nm, as shown in Fig. 1a. While the average particle size in case of *Cycas* capped AgNPs was about 35 ± 8 nm (See Fig. 1b). Moreover, in case of chitosan capped AgNPs exhibits multiple and irregular shapes with an average size in the range of 50 ± 15 nm. Finally; *Crotalaria* capped AgNPs the average size was about 30 ± 10 nm.

Colloidal Stability Properties

In addition, the colloidal properties based on DLS and zeta potential measurements for as-prepared silver nanoparticles in the presence of four different green extracts were investigated as shown in Fig. 2 and 3. It's clear that the hydrodynamic particle size of for as-prepared nanoparticles is remarkably increased, which is in agreement with their agglomeration presented in TEM micrographs as shown in Fig.1. This is due to their hydrophilicity nature. Furthermore, the strength of the steric forces of the functional groups on the surface of nanoparticles, as well as a steric interaction caused by the formation of a layer of water around the material. In this regard, *Curcumin* capped AgNPs has a hydrodynamic diameter (H_D) of 52 ± 9.1 nm (See Fig. 2a). In addition, *Curcumin* capped AgNPs exhibit a good dispersion in water, where the polydispersity index (PdI) is about 0.568. Moreover, Zeta potential (η) of *Curcumin* capped AgNPs was about -7.7 mV. While the hydrodynamic particle size of *Cycas* capped AgNPs was larger than *Curcumin* capped AgNPs of 76.57 ± 29 nm. In addition, the polydispersity index (PdI) is about 0.3 (See Fig. 2c). Moreover, Zeta potential (η) of Cycas capped AgNPs exhibits less negatively charged -11 mV. Furthermore, the hydrodynamic diameter of *Crotalaria* capped silver nanoparticles was about 160.3 ± 19.2 nm with polydispersity index (PdI) of 0.870, and the Zeta potential (η) was about -17.9 mV (See Fig. 2e). Finally, Silver capped with chitosan nanoparticles exhibited the highest H_D (Fig. 2e and Table 1). The hydrodynamic particle was about 664 ± 90.6 nm (See Fig. 2g), and the polydispersity index (PdI) is about 0.155 indicating to their fair dispersion in water. Moreover, their Zeta potential (η) was about -12.7 mV.

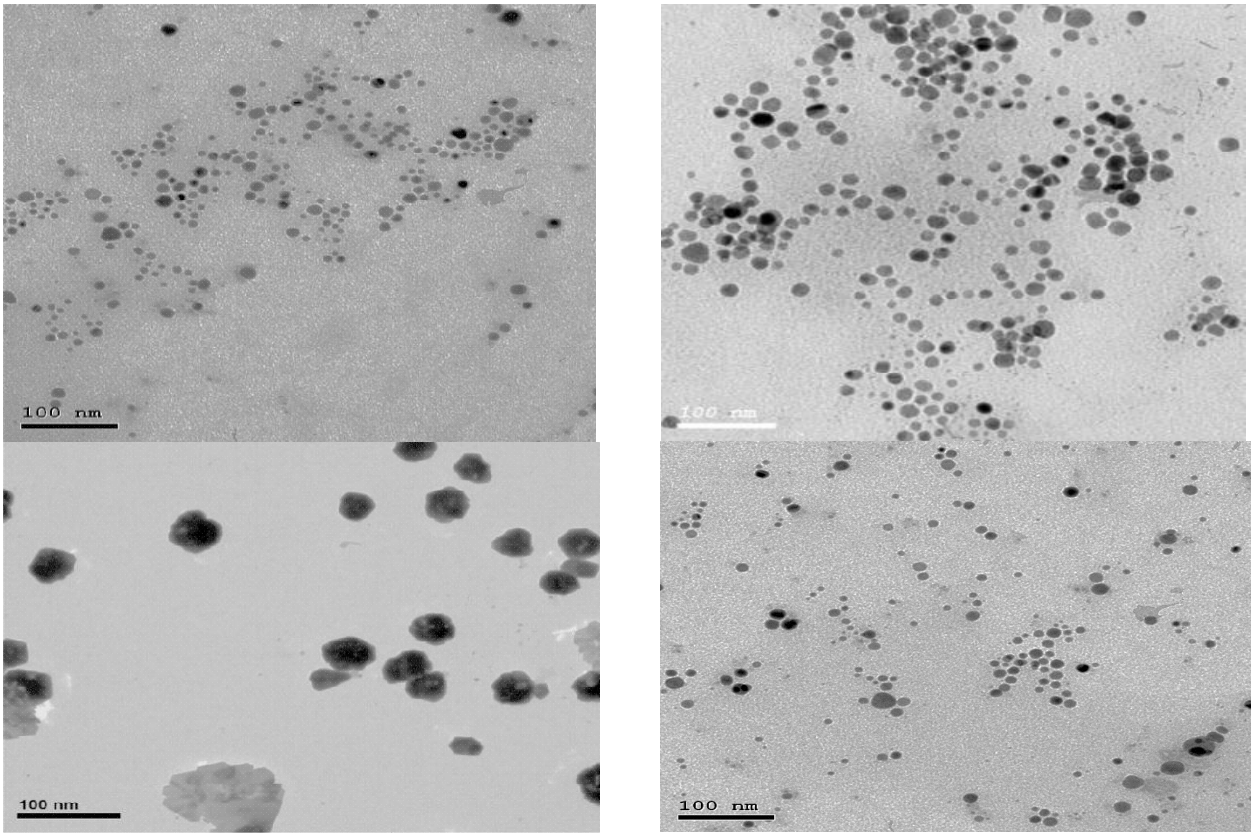

Figure (1): TEM images of as-prepared silver nanoparticles in presence of (a) *curcumin*, (b) *Cycas*, (c) chitosan and (d) *Crotalaria* extracts

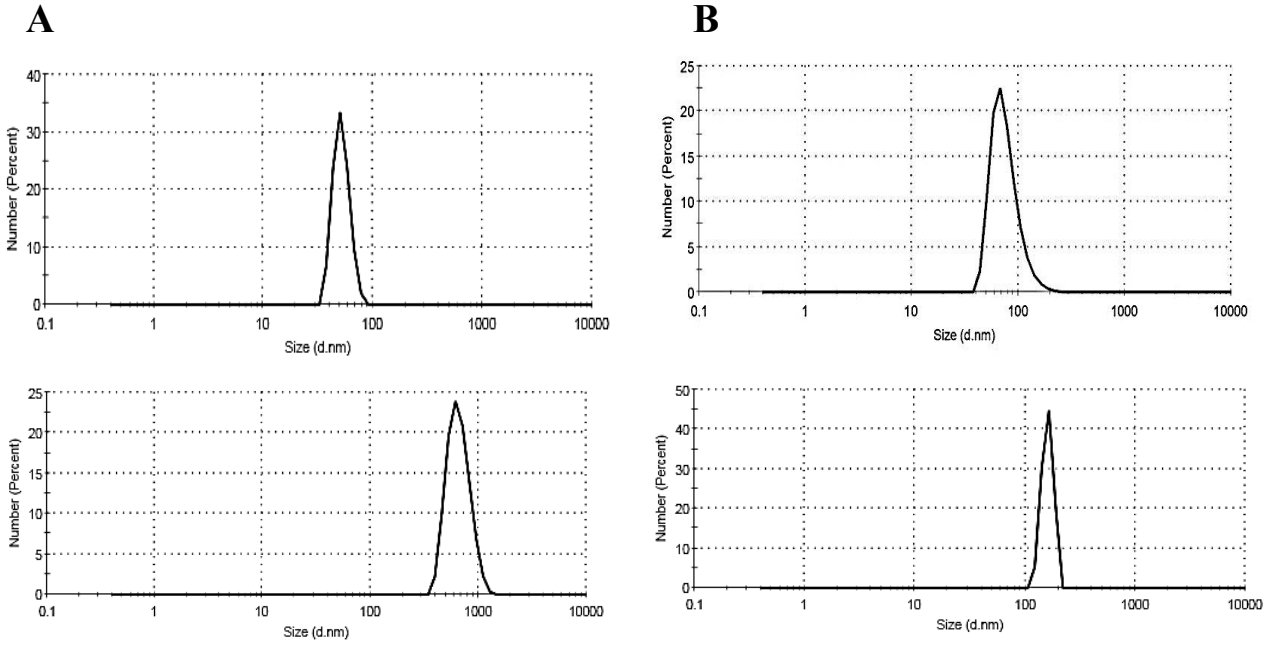

Figure (2): DLS data of as-prepared silver nanoparticles in presence of (a) *curcumin*, (b) *Cycas*, (c) chitosan and (d) *Crotalaria* extracts

Effect of AgENPs using *Curcuma comosa* on *M. incognita* and its host plant growth.

In case of *Curcuma comosa*, (N1), recorded data in Table (1) demonstrated that all the nematode parameters, e.g., galls, eggs, final population, and rate of reproduction were decreased significantly compared to infected control, the highest suppressive effect was performed using 40 ppm. followed by 20, and 10 ppm. On the other hand, data recorded

enhancing in the plant growth parameters comparing to the infected and uninfected control and the best plant growth was recorded using 40 ppm. followed by 20, and 10 ppm

Table (1): Effect of AgENPs using *Curcuma comosa* on *M. incognita* and tomato plant growth.

N1	Shoot weight	Root weight	Shoot length	Root length	fruit weight	GALLS	% RED	EGGS	% RED	PF	% RED	Rr	% RED
40 ppm	13.0a	7.1a	61.7a	24.7 abc	45.4a	0	0	0	0	0	0	0	0
	±2.3	±1.1	±1.2	±2.5	±2.9	0	0	0	0	0	0	0	0
40 ppm+ 500 IJs	12.9a	5.8abc	56.3a	24.1 abc	11.9fg	49.7de	86.0d	6601.3def	89.2f	3804.0e	82.5f	7.6d	82.5f
	±2.2	±1.6	±8.0	±1.7	±3.3	±16.6	±4.7	±315.9	±0.5	±994.3	±4.6	±2.0	±4.6
40 ppm+ 1000 IJs	11.1a	4.7 abc	51.7ab	23.3ab cd	20.2de	57.7d	89.6c	9511.0de	92.6d	4544.3de	87.3de	4.5e	87.3 de
	±1.5	±0.5	±14.0	±2.4	±1.7	±9.5	±1.7	±816.9	±0.6	±673.3	±1.9	±0.7	±1.9
40 ppm+ 2000 IJs	6.1bcd e	4.1bcd	52.5ab	25.5ab c	27.4bc	67.3d	89.6c	11063.7d	91.9e	5307.7d	86.1e	2.7ef	86.0 e
	±1.5	±0.4	±1.2	±3.0	±6.2	±4.0	±0.6	±645.3	±0.5	±164.6	±0.4	±0.1	±0.4
20 ppm	10.6a	1.0e	35.0cd e	16.2bc d	30.3b	0	0	0	0	0	0	0	0
	±0.4	±0.2	±4.6	±2.6	±5.7	0	0	0	0	0	0	0	0
20 ppm+ 500 IJs	8.2b	3.0cde	41.6 bc	20.2ab cd	18.9def	27.3ef	92.3bc	2999.0ef	95.1c	2253.0f	89.6d	4.5e	89.6 d
	±1.4	±0.2	±6.4	±6.5	±0.5	±4.0	±1.1	±142.4	±0.2	±232.2	±1.1	±0.5	±1.1
20 ppm+ 1000 IJs	5.7cdef	6.4ab	42.0bc	13.9d	20.9cd e	51.7d	90.7bc	3732.3ef	97.1b	1727.7fg	95.2bc	1.7f	95.2 bc
	±1.0	±1.4	±4.0	±2.6	±6.6	±9.1	±1.6	±443.2	±0.3	±354.9	±1.0	±0.4	±1.0
20 ppm+ 2000 IJs	7.0bc	3.9bcd	32.3cd e	15.8cd	21.2cd e	60.3d	90.7bc	4071.0def	97.0b	2102.7f	94.5c	1.1f	94.5c
	±2.1	±1.4	±12.4	±4.8	±1.2	±6.1	±0.9	±329.7	±0.2	±187.2	±0.5	±0.1	±0.5
10 ppm	4.5def	1.5de	38.3cd	26.7ab	40.8a	0	0	0	0	0	0	0	0
	±0.5	±0.2	±2.9	±6.4	±6.2	0	0	0	0	0	0	0	0
10 ppm+ 500 IJs	4.7cdef	7.4a	30.2cd e	16.3bc d	15.6ef	22.0f	93.8ab	666.3f	98.9a	455.7g	97.9ab	0.9f	97.9 ab
	±1.1	±0.8	±8.1	±3.6	±3.4	±3.6	±1.0	±83.9	±0.1	±63.1	±0.3	±0.1	±0.3
10 ppm+ 1000 IJs	5.5cdef	4.6 abc	33.2cd e	23.0ab cd	23.8bc d	23.3f	95.8a	737.0f	99.4a	576.3g	98.4a	0.6f	98.4 a
	0±.8	±2.0	±3.9	±6.5	±3.5	±3.1	±0.6	±59.9	±0.05	±35.7	±0.1	±0.09	±0.1
10 ppm+ 2000 IJs	3.3f	5.2 abc	24.7ef	25.1 abc	15.6ef	23.7f	96.3a	1032.0f	99.2a	724.3g	98.1ab	0.4f	98.1 ab
	±0.7	±1.9	±4.6	±10.7	±2.6	±3.1	±0.5	±81.2	±0.1	±100.8	±0.3	±0.1	±0.3
control - -	4.2 ef	7.1a	27.8def	28.6a	18.3def	0	0	0	0	0	0	0	0
	±1.0	±4.1	±5.8	±11.1	±3.4	0	0	0	0	0	0	0	0
control +500	4.9cdef	5.8 abc	41.1 bc	24.8 abc	14.6ef	354.7c	0	61015.3c	0	21700.3c	0	43.4a	0
	±0.7	±1.2	±7.6	±2.9	±3.7	±24.0	0	±7572.0	0	±1871.6	0	±3.7	0
control +1000	5.0cdef	4.2bcd	27.1def	24.9 abc	7.9gh	554.0b	0	129150.0b	0	35790.3b	0	35.8b	0
	±0.9	±0.1	±6.4	±1.4	±1.5	±19.3	0	±6005.6	0	±663.5	0	±0.7	0
control +2000	4.2ef	3.6bcd e	16.7f	24.4 abc	5.2h	646.0a	0	136036.7a	0	38067.7a	0	19.0c	0
	±1.0	±0.6	±2.4	±1.8	±0.8	±27.2	0	±9200.1	0	±467.9	0	±0.2	0

Means followed by the same letter(s) within a column are not significantly different at 5% level of significance, while different letters had a statistically significant differences.

Effect of AgENPs using *Cycas circinalis* on *M. incognita* and tomato host plant growth.

AgENPs in the presence of *Cycas circinalis*, data in table (2) revealed that, proportional relation between nano and suppressive effect on nematode.

The most effective nano concentration on the nematode parameters was obtained with the highest concentration (40 ppm.) In contrast, the relation between the nano concentrations and the plant growth was inversely dependent, i.e., the enhancement of the plant growth was obtained using the lowest nano concentration (10 ppm.).

Table (2): Effect of AgENPs using *Cycas circinalis* on *M. incognita* and tomato host plant growth.

N2	Shoot weight	Root weight	Shoot length	Root length	fruit weight	GALLS	% RED	EGGS	% RED	PF	% RED	Rr	% RED
40 ppm	12.5abcd	2.9d	44.9bc	23.8abc	25.0abcd	0	0	0	0	0	0	0	0
	±1.0	±1.7	±7.5	±5.7	±1.1	0	0	0	0	0	0	0	0
40 ppm+ 500 IJs	11.5bcde	5.2bcd	55.5a	21.5abc	22.7bcde	78.3e	77.9d	8544.0def	86.0g	4351.3ef	79.9d	8.7e	79.9d
	±2.9	±2.6	±5.5	±3.8	±3.9	10.6	±3.0	±648.2	±1.1	±610.3	±2.8	±1.2	±2.8
40 ppm+ 1000 IJs	13.1abcd	5.6abcd	54.5ab	23.7abc	26.5abc	115.0d	79.2cd	9493.0de	92.6d	5526.7de	0.1e	11.1d	69.1e
	±1.4	±1.0	±6.2	±3.2	±3.3	±8.0	±1.4	±945.7	±0.7	±540.8	±0.05	±1.1	±3.0
40 ppm+ 2000 IJs	13.1abcd	5.1bcd	55.5a	21.1abc	22.4bcde	121.3d	81.2c	11674.7d	91.4e	6053.3d	84.1c	3.0fg	84.1c
	±2.4	±0.4	±6.4	±8.8	±3.9	±6.1	±0.9	±810.2	±0.6	±893.9	±2.3	±0.4	±2.4
20 ppm	13.7abc	4.4bcd	54.0ab	29.6a	27.1ab	0	0	0	0	0	0	0	0
	±0.3	±0.8	±5.8	±2.9	±1.5	0	0	0	0	0	0	0	0
20 ppm+ 500 IJs	10.5de	4.4bcd	53.2ab	19.4bc	9.4hi	66.7ef	81.2c	6288.0def	89.7f	2579.3g	88.1b	5.2f	88.1ab
	±1.6	±0.7	±4.5	±3.8	±1.4	±6.7	±1.9	±809.9	±1.3	±379.5	±1.7	±0.8	±1.7
20 ppm+ 1000 IJs	9.9e	4.0d	40.3c	28.4ab	13.3gh	77.3e	86.0b	5120.3def	96.0c	2544.0g	0.1e	5.1f	85.8bc
	±2.0	±0.9	±8.1	±1.0	±2.0	±5.7	±1.0	±868.5	±0.7	±295.6	±0.1	±0.6	±1.7
20 ppm+ 2000 IJs	10.8cde	7.2ab	55.8a	18.5c	21.1cde	79.7e	87.7b	4167.3ef	96.9bc	3216.7fg	91.6a	1.6g	91.5a
	±1.2	±1.3	±2.0	±1.1	±5.9	±8.5	±1.3	±182.1	±0.1	±218.9	±0.6	±0.1	±0.6
10 ppm	10.8cde	8.1a	59.5a	23.7abc	19.9de	0	0	0	0	0	0	0	0
	±1.1	±1.5	±2.4	±3.9	±4.0	0	0	0	0	0	0	0	0
10 ppm+ 500 IJs	11.7bcde	3.4d	55.0a	23.3abc	30.2a	32.7g	90.8a	1257.7f	97.9ab	2098.7g	90.3ab	4.2f	90.3a
	±1.1	±0.7	±1.0	±4.2	±2.3	±2.5	±0.7	±177.3	±0.3	±86.6	±0.4	±0.2	±0.4
10 ppm+ 1000 IJs	14.4ab	4.9bcd	54.0ab	21.0abc	23.7bcde	39.0g	93.0a	1784.3f	98.6a	2126.0g	0.1e	4.3f	88.1ab
	±2.8	±1.1	±2.0	±3.6	±1.9	±3.6	±0.7	±201.2	±0.2	±362.9	±0.03	±0.7	±2.0
10 ppm+ 2000 IJs	14.9a	5.0bcd	63.3a	21.7abc	27.1ab	44.7fg	93.1a	2254.0ef	98.3a	3193.7fg	91.6a	1.6g	91.6a
	±1.4	±0.9	±0.6	±1.2	±3.3	±7.6	±1.2	±351.4	±0.3	±503.6	±1.3	±0.3	±1.3
control - -	4.2f	7.1abc	27.8d	28.6a	18.8ef	0	0	0	0	0	0	0	0
	±7.4	±2.3	±35.8	±11.2	±14.1	0	0	0	0	0	0	0	0
control +500	6.7f	5.8abcd	41.1c	24.8abc	14.6fg	354.7c	0	61015.3c	0	21700.3c	0	43.4a	0
	±1.0	±1.2	±7.6	±2.9	±3.7	±24.0	0	±7572.0	0	±1871.6	0	±3.7	0
control +1000	4.9f	4.2cd	16.7e	24.9abc	7.9i	554.0b	0	129150.0b	0	35790.3b	0	35.8b	0
	±0.7	±0.1	±2.4	±1.4	±1.5	±19.3	0	±6005.6	0	±663.5	0	±0.7	0
control +2000	5.0f	3.6d	27.1d	24.4abc	5.2i	646.0a	0	136036.7a	0	38067.7a	0	19.0c	0
	±0.9	±0.6	±6.4	±1.8	±0.8	±27.2	0	±9200.1	0	±467.9	0	±0.2	0

Means followed by the same letter(s) within a column are not significantly different at 5% level of significance, while different letters had a statistically significant differences

Effect of AgENPs using Chitosan on *M. incognita* and tomato host plant growth.

The recorded data in Table (3) proved the nematicidal effect of AgENPs derived using Chitosan on *M. incognita*. Moreover, Chitosan was recorded the lowest egg hatching value and the highest reduction of egg hatching, the plant growth parameters were improved by using AgENPs prepared with Chitosan and the relation was increasing proportional and the best plant growth was obtained with 40 ppm. and the lowest obtained with 10 ppm.

Effect of AgENPs using *Crotalaria juncea* on *M. incognita* and tomato host plant growth.

Data in table (4) cleared that, *Crotalaria juncea* recorded a high suppressive effect on nematode infection with the increase of the nano concentration and the best suppressive effect was obtained with the high concentration (40 ppm.) followed by 20 ppm. and 10 ppm. Our data confirm enhancement of the plant growth parameters beginning with the low concentration.

*Corresponding author e-mail: dr.entsar_elsayed@agr.asu.edu.eg
Received date 21 September 2021; revised date 27 September 2021; accepted date 03 October 2021
DOI: 10.21608/EJCHEM.2021.97189.4547

Table (3): Effect of AgENPs using Chitosan on *M. incognita* and tomato host plant growth.

N3	Shoot weight	Root weight	Shoot length	Root length	fruit weight	galls	% red	eggs	% red	Pf	% red	Rr	% red
40 ppm	12.6b	6.1bcde	60.0a	20.1bcd	19.4bc	0	0	0	0	0	0	0	0
	±2.0	±1.1	±2.0	±1.0	±4.2	0	0	0	0	0	0	0	0
40 ppm+ 500 IJs	6.3efg	7.3bc	45.0cde	14.5de	12.3de	64.3e	81.9d	6161.3def	89.9e	2175.0ef	90.0c	4.4e	90.0c
	±1.2	±1.4	±4.7	±4.2	±2.8	±3.5	±1.0	±334.0	±0.5	±136.2	±0.6	±0.3	±0.6
40 ppm+ 1000 IJs	7.3defg	4.4cde	46.9bcd	28.4a	19.3bc	95.0d	82.9cd	8914.0de	93.1d	6782.7d	81.0d	6.8d	81.1d
	±1.1	±0.1	±6.3	1.1	±3.5	7.5	±1.4	±646.9	±0.5	±657.5	±1.8	±0.7	±1.8
40 ppm+ 2000 IJs	11.4ab	2.9e	52.9abc	24.8ab	21.0ab	105.0d	83.7c	9730.3d	92.8d	7026.3d	81.5d	3.5e	81.5d
	±1.6	±0.3	±4.4	±3.0	±4.8	±7.0	±1.1	±731.1	±0.5	±182.5	±0.5	±0.1	±0.5
20 ppm	11.2ab	3.1e	48.5bcd	24.5ab	25.0a	0	0	0	0	0	0	0	0
	±0.9	±0.6	±7.4	±4.3	±1.8	0	0	0	0	0	0	0	0
20 ppm+ 500 IJs	9.9abc	12.7a	47.9bcd	16.7cde	18.5bc	58.7e	83.5cd	2332.3ef	96.2c	1358.7f	93.7b	2.7ef	93.7b
	±1.6	±4.9	±7.8	±2.3	±2.0	±4.2	±1.2	±466.5	±0.8	±308.7	±1.4	±0.6	±1.4
20 ppm+ 1000 IJs	7.7def	4.7cde	35.4ef	22.6abc	12.0de	67.3e	87.8b	3191.0def	97.5b	2365.3ef	93.4b	2.4ef	93.4b
	±0.7	±1.3	±2.4	±2.9	±3.5	±4.7	±0.9	±414.3	±0.3	±251.9	±0.7	±0.3	±0.7
20 ppm+ 2000 IJs	15.7a	8.4b	56.7ab	24.3ab	23.3ab	70.3e	89.1b	3294.7def	97.6b	2702.7e	92.9b	1.4f	92.9b
	±4.3	±1.8	±3.2	±3.7	±1.5	2.1	±0.3	±652.0	±0.5	±565.6	±1.5	±0.3	±1.5
10 ppm	9.5abcd	2.9e	55.7ab	12.7e	10.1de	0	0	0	0	0	0	0	0
	±0.9	±0.6	±1.5	±1.5	±2.4	0	0	0	0	0	0	0	0
10 ppm+ 500 IJs	11.5ab	7.0bcd	50.7abcd	20.4bcd	20.4ab	24.0f	93.2a	599.0f	99.0a	151.7g	99.3a	0.3g	99.3a
	±2.6	±0.8	±7.3	±0.9	±1.0	±4.6	±1.3	±29.8	±0.04	±15.7	±0.1	±0.06	±0.1
10 ppm+ 1000 IJs	7.4defg	7.0bcd	44.6cde	18.6bcde	23.0ab	35.7f	93.6a	668.3f	99.5a	187.0g	99.5a	0.2g	99.5a
	±2.1	±1.4	±4.9	±1.2	±2.2	±4.0	±0.7	±50.8	±0.1	±12.8	±0.07	±0.03	±0.05
10 ppm+ 2000 IJs	8.5cde	3.5de	41.0de	17.3cde	11.4de	33.0f	94.9a	1011.7f	99.3a	216.3g	99.4a	0.1g	99.4a
	±0.5	±0.6	±3.4	±2.4	±2.8	±3.0	±0.5	±84.7	±0.1	±10.7	0.0	0.0	0.0
control --	4.2g	7.1bcd	27.8f	28.6a	18.8bc	0	0	0	0	0	0	0	0
	±1.0	±4.1	±5.8	±11.1	±3.4	0	0	0	0	0	0	0	0
control +500	6.7defg	5.8bcde	41.1de	24.8ab	14.6cd	354.7c	0	±61015.3c	0	21700.3c	0	43.4a	0
	±1.0	±1.2	±7.6	±2.9	±3.7	±24.0	0	±7572.0	0	±1871.6	0	±3.7	0
control +1000	4.9fg	4.2cde	16.7g	24.9ab	7.9ef	554.0b	0	129150.0b	0	35790.3b	0	35.8b	0
	±0.7	±0.1	±2.4	±1.4	±1.5	±19.3	0	±6005.6	0	±663.5	0	±0.7	0
control +2000	5.0fg	3.6de	27.1f	24.4ab	5.2f	646.0a	0	136036.7a	0	38067.7a	0	19.0c	0
	±0.9	±0.6	±6.4	±1.8	±0.8	±27.2	0	±9200.1	0	±467.9	0	±0.2	0

Means followed by the same letter(s) within a column are not significantly different at 5% level of significance, while different letters had a statistically significant differences.

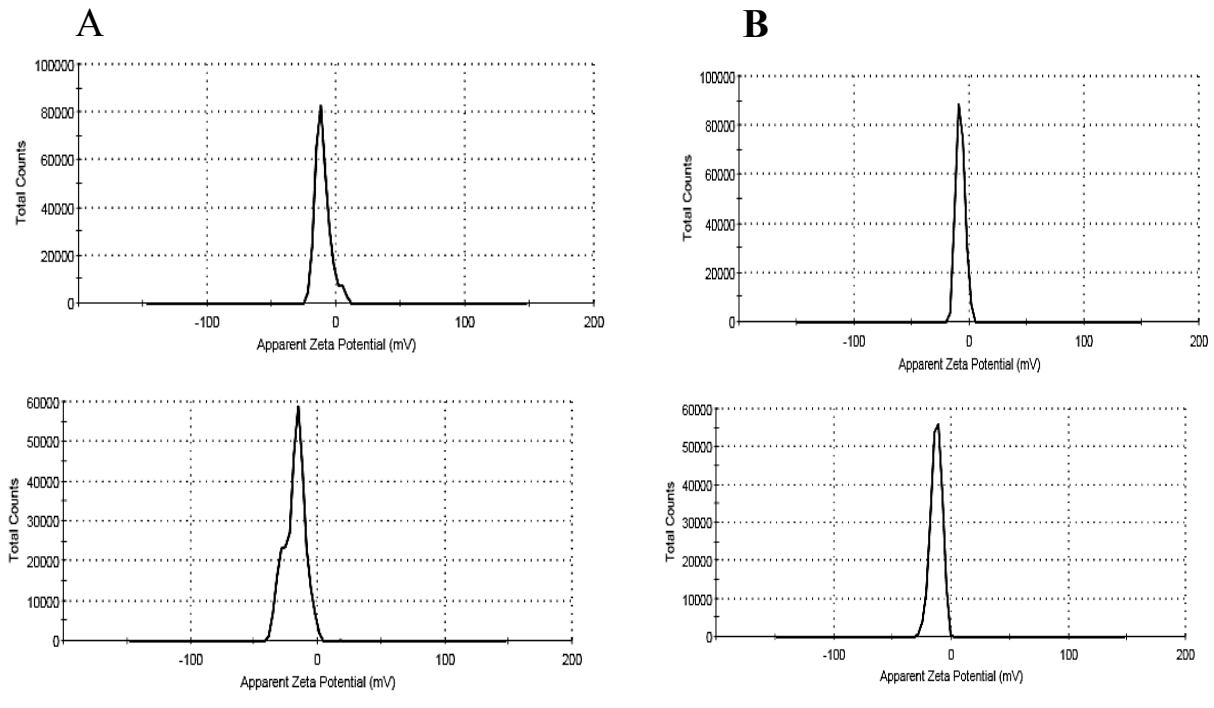

Figure (3): Zeta potential data of as-prepared silver nanoparticles in presence of (a) curcumin, (b) cycas, (c) chitosan and (d) Crotalaria extracts

Effect of AgENPs on antioxidant enzymes activity

Figure (4) showed the exchanges of the antioxidant enzyme POX, PPO, SOD, and CAT activities in plant leaves in both of infected (2000IJs) and uninfected treated plants as well as negative and positive controls. As a general trend, all the four AgENPs compounds sustained the highest values with nematode infection and the lowest values without nematodes. For SOD, data indicated that, the lowest value was recorded by uninfected control, but was equal to N3, and N4 followed by N1. The highest value was recorded in infected N2, N2, and infected control. For Catalase (CAT), data showed that, the highest value resulted from infected N2 followed by N2, infected N3, and infected control. The other treatments had low values and approximately equal.

As with, POX, data indicated that, the highest value was recorded in infected N2, followed by N2, infected N3, positive control, and infected N4. In contrast, other treatments were low in their values. For PPO, the obtained data indicated that, the highest values were arranged as, infected N2, N2, infected N3, and positive control, respectively. Other treatments were nearly equal and comparatively the lowest.

Effect of AgENPs on photosynthetic pigments

Photosynthetic pigments (chlorophyll a, chlorophyll b, carotene, and phenolic compounds) remarkably decreased as a result of nematode infection figure (5). In case of chlorophyll a results indicated that, the lowest value was 0.56 in the negative control followed by N3, infected N3, while infected and uninfected N4 were nearly alike. The lowest value was recorded in the infected control. In case of Chlorophyll b, the highest content was recorded in the treatments N3, and N4, followed by infected N3, N1, N2, positive control and infected N4. In contrast, the low data was recorded in positive control followed by infect N2 and infected N1. Regarding chlorophyll a & b all the four AgENPs compounds within the same treatment gained the highest value with nematode free and the lowest value with infected nematode. Additionally, all the four AgENPs compounds exhibited the high value with uninfected treatment than these of positive control and NPs free. Moreover, data was nearly in the same trend of chlorophyll a, and the highest value recorded was in N3 while the lowest was in positive control.

The same figure illustrates the data of carotene content recorded the highest values in the treatments N1, N2, followed by infected and uninfected N4 and negative control. While the lowest data were recorded in infected all of N1, N2, N3, uninfected N3, and positive control. Finally, total phenol content all the

*Corresponding author e-mail: dr.entsar_elsayed@agr.asu.edu.eg
Received date 21 September 2021; revised date 27 September 2021; accepted date 03 October 2021
DOI: 10.21608/EJCHEM.2021.97189.4547

four AgENPs compounds were exhibited highest value with infected nematode than positive control (uninfected NPs). The data could arrange as follow, the highest data was recorded in N4 and positive control, while infected N1, infected N2, and infected N4 were approximately equal. The lowest data were obtained with N3, N2, and negative control.

N4	Shoot weight	Root weight	Shoot length	Root length	fruit weight	GALLS	% RED	EGGS	% RED	PF	% RED	Rr	% RED
N4 1	10.1a	5.4abcd	52.7a	31.3a	15.3b	0	0	0	0	0	0	0	0
	±1.4	±1.2	±4.0	±5.7	±3.0	0	0	0	0	0	0	0	0
N4 1+1	5.9def	2.4e	37.7	24.3ab	8.9def	63.3e	82.1e	7185.7def	88.2e	4251.7f	80.4c	8.5d	80.4c
	±1.6	±0.6	±4.6	±0.6	±1.6	±3.5	±1.0	836.1	1.4	1076.1	5.0	2.2	5.0
N4 1+2	7.7cd	3.0de	44.0ab	23.3ab	8.3def	96.3d	82.6de	9493.0de	92.6d	6293.3e	82.4c	6.3de	82.4c
	±0.6	±0.6	±7.8	±2.5	±1.2	±11.0	±2.0	±945.7	0.7	1307.8	3.7	1.3	3.7
N4 1+3	7.9bcd	3.3cde	44.7ab	32.0a	6.6ef	101.0d	84.4d	11130.3d	91.8d	8026.0d	78.9c	4.0ef	78.9c
	±1.4	±0.6	±8.1	±8.9	±1.4	1±2.5	±1.9	±852.8	±0.6	±859.6	±2.3	±0.4	±2.3
N4 2	9.0abc	7.1a	47.2ab	21.1ab	21.7a	0	0	0	0	0	0	0	0
	±1.0	±1.5	±5.6	±3.0	±2.8	0	0	0	0	0	0	0	0
N4 2+1	6.8de	3.2cde	41.7ab	23.7ab	7.6ef	59.0e	83.4de	3323.3ef	94.6c	2340.3g	89.2b	4.7ef	89.2b
	±1.0	±0.9	±11.0	±3.8	±0.8	±1.7	±0.5	±588.9	±1.0	±412.8	±1.9	±0.8	±1.9
N4 2+2	6.8de	5.5abcd	46.0ab	20.2b	9.2de	63.7e	88.5c	3838.0ef	97.0b	2766.7fg	92.3b	2.8fg	92.3b
	±1.4	±1.3	±6.6	±1.8	±1.0	±4.7	±0.9	±690.6	±0.5	±781.7	±2.2	±0.8	±2.2
N4 2+3	7.9bcd	5.4abcd	41.3ab	30.3ab	9.5de	67.0e	89.6c	4167.3def	96.9b	2716.7fg	92.9b	1.4g	92.9b
	±0.8	±0.7	±6.1	±11.2	±1.7	±4.6	±0.7	±182.1	±0.1	±852.8	±2.2	±0.4	±2.2
N4 3	7.0cde	5.0abcd	36.0bc	27.7ab	11.6cd	0	0	0	0	0	0	0	0
	±1.0	±0.4	±5.6	±6.0	±0.9	0	0	0	0	0	0	0	0
N4 3+1	9.6ab	6.0ab	47.4ab	22.0ab	15.1bc	25.3f	92.9b	681.0f	98.9a	523.0h	97.6a	1.0g	97.6a
	±1.3	±0.9	±2.6	±3.0	±3.7	±2.5	±0.7	±51.6	±0.1	±44.0	±0.2	±0.1	±0.2
N4 3+2	7.0cde	5.2abcd	39.1abc	28.3ab	13.6bc	33.7f	93.9ab	784.7f	99.4a	611.7h	98.3a	0.6g	98.3a
	±0.6	±0.7	±12.0	±4.9	±1.5	±4.5	±0.8	±28.4	±0.05	±57.4	0.2	0.1	±0.2
N4 3+3	7.5cd	3.8bcde	41.7ab	29.3ab	7.8def	29.7f	95.4a	1053.7f	99.2a	795.7h	97.9a	0.4g	97.9a
	±1.7	±0.8	±9.0	±4.0	±1.4	±3.5	±0.5	±62.5	±0.05	±51.7	±0.1	±0.06	±0.1
control --	4.2f	7.1a	27.8cd	28.6ab	18.8a	0	0	0	0	0	0	0	0
	±7.4	±2.3	±35.8	±11.2	±14.1	0	0	0	0	0	0	0	0
control +1	6.7de	5.8abc	41.1ab	24.8ab	14.6bc	354.7c	0	61015.3c	0	21700.3c	0	43.4a	0
	±1.0	±1.2	±7.6	±2.9	±3.7	±24.0	0	±7572.0	0	±1871.6	0	±3.7	0
control +2	4.9ef	4.2bcde	16.7d	24.9ab	7.9def	554.0b	0	129150.0b	0	35790.3b	0	35.8b	0
	±0.7	±0.1	±2.4	±1.4	±1.5	±19.3	0	±6005.6	0	±663.5	0	±0.7	0
control +3	5.0ef	3.6bcde	27.1cd	24.4ab	5.2f	646.0a	0	136036.7a	0	38067.7a	0	19.0c	0
	±0.9	±0.6	±6.4	±1.8	±0.8	±27.2	0	±9200.1	0	±467.9	0	±0.2	0

Table (4): Effect of AgENPs using *Crotalaria juncea* on *M. incognita* and tomato host plant growth

Means followed by the same letter(s) within a column are not significantly different at 5% level of significance, while different letters had a statistically significant differences.

Effects of AgENPs on silver residue in tomato fruits

Generally, Ag nano accumulation in tomato fruits after application is relatively lower. Estimation of AgENPs residue in tomato fruit indicated that, the highest value was 0.014 mg/kg and recorded by N2 of (40 ppm.) followed by 0.012 mg/kg which detected by N2 (20 ppm.) As a general, the lowest value of nano silver residue was exhibited by N3, in all concentrations; 0.002, 0.002 and 0.004 mg/kg with 10, 20, 40 ppm. respectively (fig. 6).

As with DNA damage, DNA in tail, tail length, tail moment and olive tail moment are accurate parameters to detect the effect of AgENPs compounds on DNA in the cell. Generally, the data in Table (5) demonstrated that, the effect of AgENPs on

DNA damage increased proportionally with the concentration of AgENPs.

Table (5): Estimation of DNA Damage in roots of tomato treated with three concentrations of four AgENPs by comet assay.

Treatments		Damage %	Tail length	DNA in tail	Tail moment	Olive tail moment
AgNPs	Conc. ppm					
Curcuma comosa	10	10.2	9.35	7.87	0.82	1.34
	20	12.3	5.70	8.64	0.40	1.09
	40	18.6	9.66	8.15	0.85	1.39
Cycas circinalis	10	12.6	6.94	8.24	0.56	1.25
	20	15.7	6.05	12.07	0.81	1.55
	40	16.5	8.27	8.89	0.73	1.44
Chitosan	10	10	6.56	8.03	0.49	0.94
	20	10.7	6.41	9.91	0.66	1.28
	40	16.6	7.23	9.17	0.61	1.15
Crotalaria juncea	10	11.4	5.07	8.16	0.42	1.01
	20	11.6	6.60	8.67	0.54	1.21
	40	18.8	7.55	9.07	0.67	1.15
Control	0	3.64	2.22	2.61	0.19	0.32

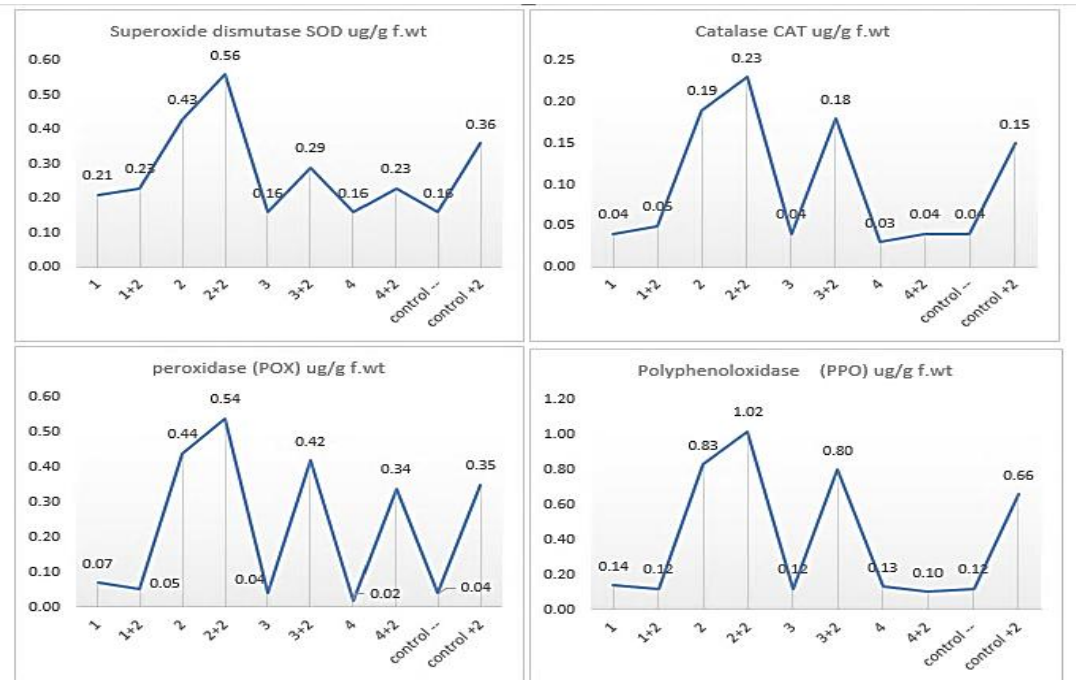

Figure (4): Effect of AgENPs on antioxidant enzymes activity in tested plants. *Curcuma comosa* (N1), *Cycas circinalis* (N2), Chitosan (N3), and *Crotalaria juncea* (N4), +2 mean infected with 1000 IJs.

Figure (5): Effect of four AgENPs on photosynthetic pigments in tested tomato plants, +2 mean infected with 1000 IJs.

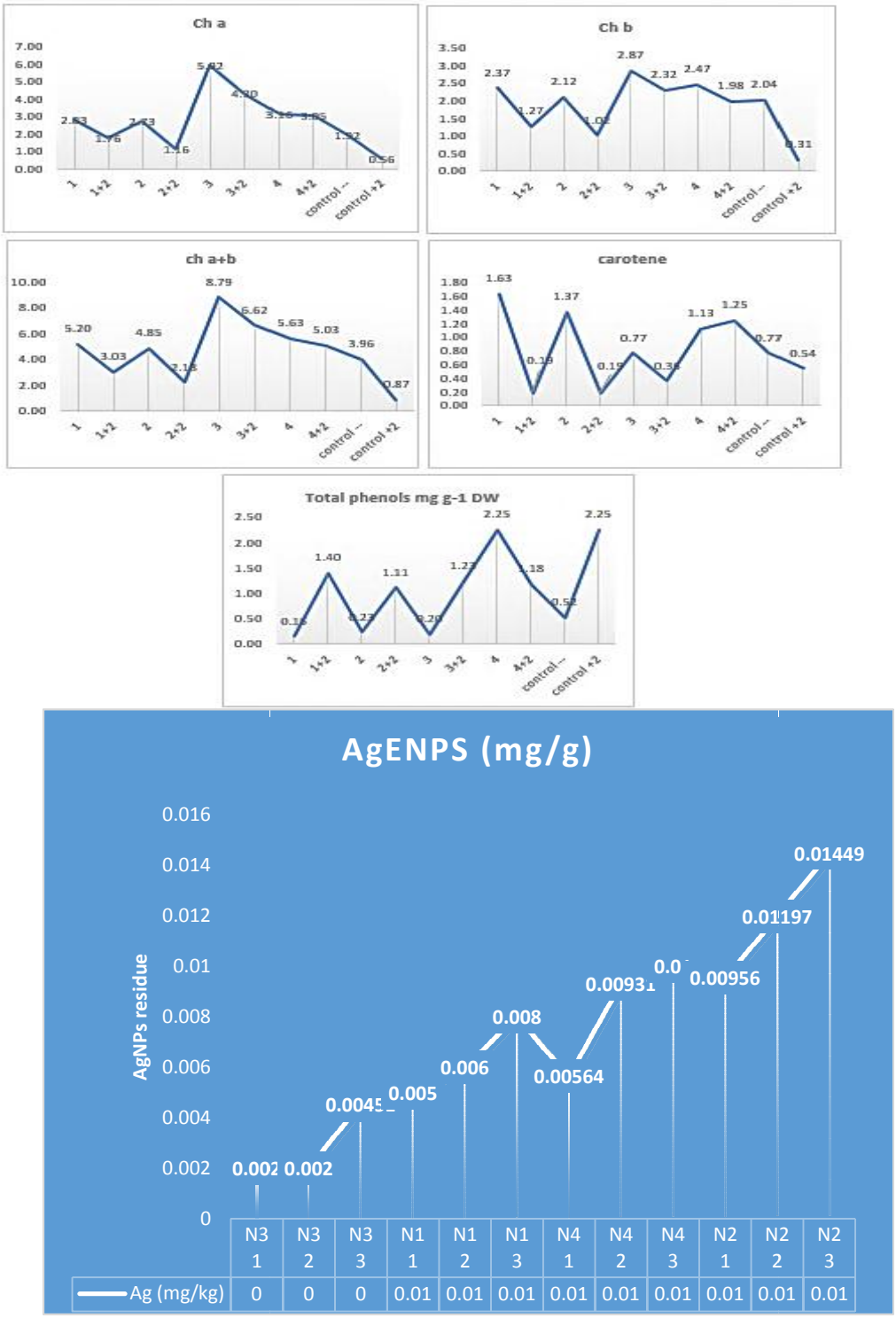

Figure (6): Effect of AgENPs on silver residue in tomatoes fruits

By using *Curcuma comosa* it was found that, the lowest DNA damage (10.2 %) was recorded with 10 ppm and the highest percentage was detected with 40 ppm (14.6%). The lowest tail length was 5.7 pixel observed with 20 ppm while the highest tail length was 9.66 pixel appeared with 40 ppm. As with, the percentage of DNA in the tail of comet, the lowest percentage 7.87 % was detected with 10 ppm and the highest percentage was observed with 20 ppm (8.64 %). Moreover, the lowest tail moment (0.40) appeared with 20 ppm and the highest one 0.85 was detected with 40 ppm. For the olive tail moment, the

lowest value 1.09 was recorded with 20 ppm while the highest value (1.39) was exhibited with 40 ppm.

Cycas circinalis indicated that, the lowest percentage of DNA damage was 12.6 % with 10 ppm and the highest one was 16.5 % was performed with 40 ppm. The lowest tail length was 6.05 pixel appeared with 20 ppm and the highest was 8.27 pixel exhibited with 40 ppm. For the percentage of DNA in the tail of comet, the lowest percentage was 8.24 % which detected with 10 ppm, and the highest percentage was 12.07 % with 20 ppm. However, the lowest tail moment was 0.56 appeared with 10 ppm and the highest value 0.81 was recorded with 20 ppm. For the olive tail moment, the lowest value 1.25 was recorded with 10 ppm while the highest one 1.55 was exhibited with 20 ppm.

As with ***Crotalaria juncea*** it was found that, the lowest DNA damage 11.4 % was recorded with 10 ppm while the highest percentage 18.8 % was detected with 40 ppm. The lowest tail length (5.07 pixel) was observed with 10 ppm while the highest tail length (7.55 pixel) was exhibited with 40 ppm. The lowest percentage of DNA in tail of comet 8.16 % was indicated with 10 ppm but the highest percentage 9.07 % was observed with 40 ppm. Moreover, the lowest tail moment 0.42 was appeared with 10 ppm and the highest one 0.67 was detected with 40 ppm. For the olive tail moment, the lowest value 1.01 was performed with 10 ppm while the highest value 1.21 was exhibited with 20 ppm. The present results of comet analysis of DNA damage were important in determining the concentration of AgENPs required for applying with *M.incognata*.

DISCUSSION

In case of *Curcuma comosa*, (N1), data demonstrated that all the nematode parameters were decreased significantly compared to infected control, these data were agreed with **Goel *et. al.,*[9]; Neeraj et.al.,[42];** and **Rashid *et. al.,*[43]** who reported suppressive effect on nematode activity and pathogenicity, using of *Curcuma comosa* which may be due to its high containing of nematicidal compounds on plant parasitic nematodes, such as alkaloids, diphenylheptanoids, phenolics, fatty acids, iso-thiocyanates, and thiophenics without any hazard effect on non-target organisms[5,43,44,45]. On the other hand, the enhancing in the plant growth parameters agreed with **Yasmeen *et. al.*,[46] and Hojjat[47]** who attributed the extending suppressive effect of AgNPs to other plant parasitic nematodes as well as plant microbial pathogens which induce immunity in the infected plants and in turn, increased in the plant growth[48,49,50]**.**

AgENPs in the presence of *Cycas circinalis*, revealed to a proportional relation between nano and suppressive effect on nematode, and this result supported by **Logeswari *et. al.,*[3]; Johnson & Prabu[20]** In contrast, the relation between the nano concentrations and the plant growth was inversely dependent, as explained before *Cycas* plant itself has no effect on the nematode infectivity. However, the highly reductive power of Cycas in the nano synthesis may be due to the infinite small particles which produce more efficient in the penetration into the plant cells[3].

Our recorded data proved the nematicidal effect of AgENPs derived using Chitosan on *M. incognita* which is in accordance with **Bernard *et. al.,*[51]**. The free radicals which developed by AgENPs surface can cause membrane damage and oxidative stress which provide more suppressive effect of the nano particles[52]. While, the highest reduction of egg hatching, this effect may be due to the hydrolysis effect of chitin which cause the eggshell lysis and inhibit egg hatching and subsequently the emerged larvae were immediately[53]. The plant growth parameters were improved by using AgENPs prepared with Chitosan increased proportionally this enhancement of plant growth may be due to the inducing of the defense hormones in the treated plant. On the other hand, the high dose causes an accumulation of IAA in the plant roots and depolarize the plasma membrane in the root plant cells which increase the cell penetration[54] and increasing some oxylipins, that are essential for the plant resistance to nematodes[55,56]**.**

Data of using of *Crotalaria juncea* recorded a high suppressive effect on nematode infection with the increase of the nano concentration and these data agreed with those of **Taha[8]; Thoden *et. al.*,[18],** which may attribute to their highly content of Pyrrolizidine alkaloids (PAs) which recorded extra decreasing effect on nematode development as well as their life cycle[18,53]**.** In addition to that PAs has a harm affect other plant pathogens which mean the improvement of plant growth[18]**.**

As a general trend, all the four AgENPs compounds caused exchanges of the antioxidant enzyme POX, PPO, SOD, and CAT activities in plant leaves, moreover, the highest values with nematode infection and the lowest values without nematodes. Increasing of antioxidative enzymes in our results were approved by **Song et. al.,[12]**; **Dolenc Koce[57]** using Zn nano particles and**.,** in velvet mesquite plant and in soya bean plants by **Hernandez et al[58] Lu et al.[59]; Kim et al. [60]** using a mixture of nano-SiO_2 and nano-TiO_2. SOD can convert O_2 into H_2O_2 and O_2; moreover, CAT, APX, and GR can reduce H_2O_2 into H_2O and O_2. Therefore, SOD, CAT, and APX can maintain a low level of reactive oxygen species (ROS) and prevent ROS toxicity that protect cells[55]**.** On the other hand, nano particles induced can increasing of antioxidative enzymes which may represent a secondary defensive mechanism against oxidative stress which is not as direct as primary defensive responses. At chronic nanoparticle

concentrations of ZnO, antioxidative mechanisms seem to operate in an additive way to overcome effectively with nanoparticle stress[64]. Therefore, the induction of CAT, APX, SOD and GR provides additional defense against metal toxicity and keeps the metabolic activities in plant functionally.

photosynthetic pigments (chlorophyll a, chlorophyll b, carotene, and phenolic compounds) recorded remarkable decrease in our results which confirmed by **Song et al.,[12] and Zheng *et al.*,[62]** attributed this reaction may be due to that nanoparticles NPs can induce reactive oxygen species production, which result in oxidative stress and cause an increase in antioxidative enzymes. SOD and CAT are important protective enzymes of antioxidant defense systems, through which plant can eliminate H_2O_2, O_2 and other reactive oxygen species[63,64].

Ag nano accumulation in tomato fruits after application is relatively lower. Such conclusion supported where roots are coming in direct contact of ENPs and therefore shows the aggregation on the root surfaces. Transport barriers also may play a crucial role in nanoparticle translocation.

The silver ions residue in the plant tissue is correlated to the AgNPs concentration, the plant age and the application method[65]. To eliminate and get rid out of this residue is by accumulating these ions in the old leaves and drop it fall. Moreover, remain residues is a highly small amount that is less in the plant fruit which is the most important eaten part in whale plants[66].

The present results of comet analysis of DNA damage were important in determining the concentration of AgENPs required for applying with *M.incognata*. As with DNA damage, DNA in tail, tail length, tail moment and olive tail moment are accurate parameters to detect the effect of AgENPs compounds on DNA in the cell and this is agreed with **Sun *et al.*,[10]** and **Fabbrizi *et al*,[21]** who demonstrated that, enzyme-modified neutral comet (EMNC) assay can be used clearly to quantify the levels of complex DNA damage (CDD) in the presence and absence of NPs DNA damage agents for their capability to induce CDD. Significant DNA damage was induced by all concentrations of SiO_2NPs compared to control group[26]. Comet assay was performed to detect the genotoxicity of triclocarban 3,4,4′-trichlorocarbanilide, TCC[10]. Other analytical limitations of the neutral comet assay in plants relate to migration of DNA under neutral conditions: often the differences depend on the size of plant genomes and protein content[67]. Furthermore, the advantages of this assay include its simplicity (small cell analysis), fast performance, and high sensitivity to various types of DNA damage in any cell type, as long as nucleated[68]. Quantitative improvement is required to reveal the full potential of the comet assay and enhance its role in the battery of in vivo approaches to characterize determination of safe human exposure limits[22].

CONCLUSION

The green synthesis of nano silver using plants which have nematicidal properties on plant parasitic nematodes such as *Curcuma comosa*, *Crotalaria juncea*, or natural nematicidal degradable compound such as Chitosan, as well as an efficient reductive plant such as *Cycas circinalis*, may introduce an alternative method to control plant parasitic nematodes and improve plant growth using low concentrations to avoid any side effect of the chemical reductive agents which use in the AgNPs synthesis.

Acknowledgements Authors wishes thanks to everyone helping to do this study .

Funding This study funded by National Research Center, Dokki, Cairo, Egypt, Project No. 12020230.

REFERENCES

1. Katiyar P., Yadu B., Korram J., Satnami M.L., Kumar M. & Keshavkant S.: Titanium nanoparticles attenuates arsenic toxicity by up-regulating expressions of defensive genes in *Vigna radiata* L. Journal of environmental sciences, 92, 18-27, 2020
2. Kalaiselvi D., S. Palanisamy & S. Hafez: Nematicidal activity of green synthesized silver nanoparticles using plant extracts against root-knot nematode *Meloidogyne incognita*. International Journal of Nematology, 27(1) : 81-94, 2017.
3. Logeswari, P., S. Silambarasan & F. Abraham: Ecofriendly synthesis of silver nanoparticles from commercially available plant powders and their antibacterial roperties. Scientia Iranica J. 20 (3), 1049– 1054, 2013. http://dx.doi.org/ 10.1016/j.scient.2013.05.016
4. Kayani, M.Z., Mukhtar, T. & Hussain, M.A.: Interaction between nematode inoculum density and plant age on growth and yield of cucumber and reproduction of Meloidogyne incognita. Pakistan J. Zool., 50: 897-902, 2018. https://doi.org/ 10.17582/ journal.pjz/ 50. 3.897.902
5. Abdellatif K.F., Ragaa A. Hamouda & El-Ansary, M.S.M.. Green Nanoparticles Engineering on Root-knot Nematode Infecting Eggplants and Their Effect on Plant DNA Modification. Iran J Biotech. 14(4): 3-9, 2016. DOI:10.15171/ijb.1309.
6. Mallaiah, B.: Integrate approaches for the management of Crossandra (*Crossandra infundibuliformis* L. nees) wilt caused by *Fusarium incarnatum* (Desm.) Sacc. PhD Thesis. Madurai, India: Tamil Nadu Agric. Univ. 2015. http://hdl.handle.net/10603/234713
7. Chen J, Wang X & Han H: A new function of graphene oxide emerges: inactivating

phytopathogenic bacterium, Xanthomonas oryzae pv. oryzae. J Nanopart Res, 15:1658, 2013.
8. Taha, H. Entsar: Nematicidal Effects of Silver Nanoparticles on Root-knot Nematodes (*Meloidogyne incognita*) in laboratory and screenhouse. *J. Plant Prot. and Path.,* Mansoura Univ., 7 (5): 333 - 337, 2016.
9. Goel N., S.R., Anil Kumar, Gurpreet Singh & V.K.Madan: Effect of Plant Extracts on Hatching and Mortality of Root-Knot Nematode, *Meloidogyne incognita* Larvae (*in-vitro*). *Biosci., Biotech. Res. Asia,* 14(1), 467-471, 2017
10. Sun, D., T. Zhao, T. Wang, M. Wu & Z. Zhang: Genotoxicity assessment of triclocarban by comet and micronucleus assays and Ames test. *Environmental Science and Pollution Research.* 27:7430–7438, 2020. https://doi.org/10.1007/s11356-019-07351-9
11. Atha D.H., Wang H., Petersen E.J., Cleveland D., Holbrook R.D., Jaruga P., Dizdaroglu M., Xing B. & Nelson B.C. *Models* Copper Oxide Nanoparticle Mediated DNA Damage in Terrestrial Plant. *Environ Sci Technol.*;46:1819-1827, 2012. DOI:10.1021/ es202660k.
12. Song, U., H. B. Waldman, J. Roh, Y. Kim, J. Yi and E. Lee: Functional analyses of nanoparticle toxicity: Acomparative study of the effects of TiO_2 and Ag on tomatoes (*Lycopersicon esculentum*) Ecotoxicology and Environmental Safety 93; 60–67, 2013.
13. Gudikandula, K. &Maringanti, S.C.: Synthesis of silver nanoparticles by chemical and biological methods and their antimicrobial properties. J. Exp. Nanosci. 11, 714–721, 2016.
14. Willner, I., Basnar, B. & Willner, B.: Nanoparticlee enzyme hybrid systems for nanobiotechnology. FEBS J. 274, 302-309, 2007. https://doi.org/10.1111/j.1742-4658.2006 .05602.x.
15. Kumar, P.S.M., Francis, A.P. & Devasena, T.: Comparative studies on green synthesized and chemically synthesized titanium oxide nanoparticles. A validation for green synthesis protocol using Hibiscus flower. J. Environ. Nanotechnol. 3, 78-85, 2014. https://doi.org/ 10.13074/jent.2014.12.144115
16. Escudero, De la Torre- E, Bennett A.P.S., Clarke A, Brennan GP & Robinson M.W.: Extracellular vesicle biogenesis in helminths: more than one route to the surface? Trends Parasitol 32:921–929, 2016. https://doi. org/10.1016/j.pt.2016.09.001
17. Fan, Z., Yukun, Q.; Song L.; Ronge X.; Huahua Y.; & Pengcheng L.: Chitosan Oligosaccharide Fluorinated Derivative Control Root-Knot Nematode (*Meloidogyne incognita*) Disease Based on the Multi-Ecacy Strategy. Mar. Drugs. 18, 273, 2020. doi:10.3390/md18050273
18. Thoden, T. C, Michael B. & Johannes H.: Effects of pyrrolizidine alkaloids on the performance of plant-parasitic and free-living nematodes. Pest Manag Sci.; 65: 823–830, 2009. DOI 10.1002/ps.1764
19. Cromwell, W. A., Yang, J., Starr, J. L. & Jo, Y. K. Nematicidal effects of silver nanoparticles on root-knot nematode in bermuda grass. Journal of Nematology 46:261–6, 2014.
20. Johnson, H. & J. Prabu: Green synthesis and characterization of silver nanoparticles by leaf extracts of Cycas circinalis, Ficus amplissima, Commelina benghalensis and Lippia nodiflora. Int Nano Lett 5:43–51, 2015. DOI 10.1007/s40089-014-0136-1
21. Fabbrizi M. R., Jonathan R. H. & Jason L. P.: The Enzyme-Modified Neutral Comet (EMNC) Assay for Complex DNA Damage Detection Methods Protoc., 4-14. 2021. https://doi.org/10.3390/mps4010014
22. Cordelli, E., Margherita B. & Pacchierotti F.: Comet assay: a versatile but complex tool in genotoxicity testing.*Toxicology Research*, 10(1); 68–78, 2021. https: //doi.Org/10.1093/toxres/ tfaa093
23. Lan, P. & Gray, J.: Predicting effects of toxic chemicals in the marine environment. Marine Pollution Bulletin, 42(3), 169–173, 2003.
24. Van Der Oost, R., Beyer, J. & Vermeulen, N.P.E.: Fish bioaccumulation and biomarkers in environmental assessment: a review. Environmental Toxicology and Pharmacology. 13(2); 57-149, 2003. http:// dx.doi.org/10.1016/S1382-6689(02) 00126-6.
25. Jemec, A., Drobne, D., Tisler, T., Trebse, P., Ros, M., & Sepcic, K.: The applicability of acetylcholinesterase and glutathione S-transferase in Daphnia magna toxicity test. Comparative Biochemistry and Physiology, Part C, 144, 303–309, 2007.
26. Liman, R., Acikbas, Y., Hakkı İ. C., Ali M.M. & Kars M.D.: Cytotoxic and Genotoxic Assessment of Silicon Dioxide Nanoparticles by Allium and Comet Tests. *Bulletin of Environmental Contamination and Toxicology* 104:215–221, 2020. https://doi.org/ 10.1007/s00128-020-02783-3
27. Mukherjee, S.P. & Choudhuri, M.A.: Implication of water stress-induced changes in the level of endogenous ascorbic acid and hydrogen peroxide in *Vigna* seedlings. Pjysiol. Plant. 58:166-170, 1983.
28. Marklund, S. & Marklund, G.: Involvement of the superoxide anion radical in the autoxidation of pyrogallol and a convenient assay for superoxide dismutase. Eur. J. Biochem . 47, 469-474, 1974.
29. Kong, F.X., Hu, W., Chao, S.Y., Sang, W. L. & Wang, L.S.: Physiological responses of mexicana to oxidative stress of SO_2. Environ and Exp. Bot. 42: 201-209, 1999.

30. Chen, Y., Cao, X.X.D., Lu, Y. & Wang, X.R.: Effect of rare earth metal ions and their EDTA complex on antioxidant enzymes of fish liver Bull. Environ. Contam. Toxicol.65:357-365, 2000.
31. Bergmeyer, H.U.: Methods of Enzymatic Analysis. Seacond english eddition. Acadimic press. New York Volume 4;1831-1835,1974.
32. Kar, M. & Mishra, D.: Catalase, peroxidase and polyphenol oxidase activities during rice leaf senescence. Plane physiol.57:315, 1976.
33. Razinger, J., Dermastia M., Drinovec, L., Drobne, D., Zrimec, A. & Dolenc Koce J.: Antioxidative responses of duckweed (*Lemna minor* L.) to short-term copper exposure. Environ. Sci. Pollut. R. 14, 194-201, 2007.
34. Vernon, L.P. & Seely, G.R.: The chlorophylls. Acad. Press, New York, London, 1966.
35. Lichtenthaler, H.K.: Chlorophylls and carotenoids, the pigments of photosynthetic biomembranes. In: Douce, R. and Packer, L. (eds.), Methods Enzymol. 148, 350-382, 1987. Academic Press Inc., New York.
36. Thomas, R.L., Sheard, R.W. & Moyer, J.R.: Comparison of conventional and auto- mated procedures for nitrogen, phosphorus and potassium analysis of plant materials usinga single digestion. Agronomy J., 59(3), 240- 243, 1967. doi:10.2134/agronj1967.00021962005900030010x
37. NIST: Report of Investigation, Reference Material 8437 Hard Red Spring Wheat Flour., Document, National Institute of Standards and Technology, Gaithersburg, MD, USA, 2008
38. Georgieva, M. & Stoilov L.: Assessment of DNA strand breaks induced by bleomycin in barley by the comet assay. Environmental and Molecular Mutagenesis 49(5):381-387, 2008. DOI:10.1002/ em. 20396
39. Parrella A., M. Lavorgna, E. Criscuolo, C. Russo & M. Isidori: Eco-genotoxicity of six anticancer drugs using comet assay in daphnids. Journal of Hazardous Materials 286: 573–580, 2015.
40. Snedecor, G.W., & Cochran, W.G.: Statistical methods. 8th ed. Iowa State University Press, 1989
41. Duncan, D. B.: Multiple range and multiple, F-test. *Biometrics, 11*, 1-42, 1955. https:// doi.org/ 10.2307/ 3001478
42. Neeraj, G. S., A. Kumar, S. Ram & V. Kumar: Evaluation of Nematicidal Activity of Ethanolic Extracts of Medicinal Plants to *Meloidogyne incognita* (Kofoid and White) Chitwood under Lab Conditions. Int. J. Pure App. Biosci. 5 (1): 827-831, 2017. DOI: http://dx.doi.org/10.18782/2320-7051.2525 ISSN: 2320 – 7051.
43. Rashid, U., Aijaz P., Aysha F., Musarrat A., Nusrat J. & Durdana R. H.: Nematicidal Effects of Various Fractions of Curcuma longa against *Meloidogyne incognita* (root knot nematodes). Turk J. Agr Eng Res, 2(1), 175-182, 2021. https://doi.org/10.46592/ turkager.v02i01.013.
44. Prasad, D. & N. Suverna: Nematicidal toxicity of Calotropis procera and Parthenium hysterophorus extracts on pre-adults of Rotylenchulus reniformis. Annals of Plant Protection Sciences 13 (2), 445-449, 2005.
45. Baronia, R., P. Kumar, S. P. Singh & R. K. Walia: Silver nanoparticles as a potential nematicide against *Meloidogyne graminicola*. J. of Nematolgy;52:1-9,2020. doi: 10.21307/jofnem-2020-002.
46. Yasmeen, F., Razzaq, A., Iqbal, M.N. & Jhanzab , H.M.: Effect of silver, copper and iron nanoparticles on wheat germination. Int. J. Biosci., 6: 112-117, 2015. https://doi.org/10.12692/ ijb/ 6.4.112- 117
47. Hojjat, S.S.: The effect of silver nanoparticle on lentil seed germination under drought stress. Int. J. Farm. Allied Sci., 5: 208-212:2016.
48. Nazir K., T. Mukhtar & H. Javed: In Vitro Effectiveness of Silver Nanoparticles against Root-Knot Nematode (*Meloidogyne incognita*) Pakistan J. Zool., 51(6); 2077-2083, 2019. DOI: ttp://dx.doi.org/10.17582/ journal.pjz/2019.51.6.2077.2083.
49. Lim D, Roh JY, Eom HJ, Choi JY, Hyun J. & Choi J.: Oxidative stress related PMK 1 P38 MAPK activation as a mechanism for toxicity of silver nanoparticles to reproduction in the nematode, Caenorhabditis elegans. Environmental Toxicology and Chemistry. 31(3): 585-592, 2012.
50. Ahamed, M., Posgai, R., Gorey, T.J., Nielsen, M., Hussain, S.M. & Rowe, J.J.. Silver nanoparticles induced heat shock protein 70, oxidative stress and apoptosis in *Drosophila melanogaster*. *Toxicology and Applied Pharmacology, 242*, 263-269, 2010. https://doi.org/ 10.1016/ j.taap.2009.10.016
51. Bernard, G. C., Jacob F., Byungjin M., Naresh S., Marceline E., Inocent R., Willard E. C., & Conrad B.: Potential nematicidial activity of silver nanoparticles against the root-knot nematode (*M. Incognita*). Journal of Complementary & Alternative Medicine: 2(2), 1-4, 2019. DOI: 10.33552/OJCAM.2019 .02 .000531.
52. Khalil M.S. & Badawy M.E.I.: Nematicidal activity of a biopolymer chitosan at different molecular weights against root-knot nematode, *Meloidogyne incognita*. Plant Protect Sci 48(4): 170-178,2012.
53. Wang, L.F.; Habibul, N.; He, D.Q.; Li, W.W.; Zhang, X.; Jiang, H. & Yu, H.Q.: Copper release from copper nanoparticles in the presence of natural organic matter. Water Res., 68, 12–23, 2015.
54. Suarez- Fernandez. M., Marhuenda-Egea, F. C., Lopez-Moya, F., Arnao, M. B., Cabrera-Escribano, F. & Nueda, M. J.: Chitosan Induces

Plant Hormones and Defenses in Tomato Root Exudates. Front. Plant Sci. 11:572087, 2020. doi: 10.3389/fpls.2020.572087.
55. Vasyukova, N. I., Zinov'eva, S. V., Udalova, Z. V., Panina, Y. S., Ozeretskovskaya, O. L., & Sonin, M. D.: The role of salicylic acid in systemic resistance of tomato to nematodes. Dokl. Biol. Sci. 391, 343–345, 2003. doi: 10.1023/A: 1025158702692
56. Singh, M., Poddar, N. K., Singh, D., & Agrawal, S.: Foliar application of elicitors enhanced the yield of with anolide contents in *Withania somnifera* (L.) Dunal (variety. Poshita). 3 Biotech 10:157, 2020. doi: 10.1007/s13205-020- 2153-2
57. Dolenc Koce, J.: Effects of exposure to nano & bulk sized TiO2 and CuO in Lemna Minor. Plant Physiology and Biochemistry 119 43-49, 2017. http://dx.doi.org/ 10.1016/j.plaphy.2017.08.014
58. Hernandez,V., J.A., H. Castillo-Michel, A.D. Servin, J.R. Peralta-Videa & J.L. Gardea-Torresdey: Spectroscopic verification of zinc absorption and distribution in the desert plant Prosopis juliflora-velutina (velvet mesquite) treated with ZnO nanoparticles, Chem. Eng. J. 170;346–352, 2011.
59. Lu, C.M., C.Y. Zhang, J.Q. Wen & G.R. Wu: Effects of nano material on germination and growth of soybean, Soybean Sci. 21; 168–171, 2002.
60. Kim, S. W., Sun H. & Youn J.: Interaction of Silver Nanoparticles with Biological Surfaces of *Caenorhabditise legans*. Ecotoxicology and Environmental Safety; 77; 64–70, 2012. doi:10.1016/j.ecoenv. 2011.10.023.
61. Rao, S. & G.S. Shekhawat: Toxicity of ZnO engineered nanoparticles and evaluation of their effect on growth, metabolism and tissue specific accumulation in Brassica juncea. Journal of Environmental Chemical Engineering (2) 105–114, 2014. http://dx.doi.org/10.1016 /j.jece.11.029
62. Zheng L., Liu D., Lu F.S. & Liu C.: Effect of Nano TiO_2 on strength of naturally aged seed and growth of spinach. Biological Trace Element Research, 104:83-91, 2005.
63. Wang, B.S.: Biological free radicals and membrane damage of plants (in Chinese). Plant Physiol. Commun. 2 12–16, 1988.
64. John G. & J.G. Scandalios: Oxygen stress and superoxide dismutase, Plant Physiol. 101: 7–12,1993.
65. Kafi M., Borzooei A., Salehi M., Kamandi A., Maasoomi A. & Nabati J.: Physiology of environmental stresses in plants. Jahad University Publication, Mashhad, pp 502, 2010
66. Shams, G. & M. Ranjbar: Effect of silver nanoparticles on concentration of silver heavy element and growth indexes in cucumber (*Cucumis sativus* L. Negeen). J Nanopart Res 15:1630, 2013. DOI: 10.1007/s11051-013-1630-5
67. Armalytė J. & Žukas K.: Investigation of UVC-induced DNA damage and its repair by SCGE assay in barley. *Ekologia* (Vilnius), 1: 72-74,2003.
68. Brianezi, G., de Camargo J.L.V. & Miot H.A.: Desenvolvimento e validação de técnica quantitativa de análise de imagem para avaliação do teste do cometa corado pela prata. *Jornal Brasileiro de Patologia e Medicina Laboratorial,* 45(4), pp. 325-334, 2009.