Nematicidal Activity of Chemical and Green Biosynthesis of Copper Nanoparticles Against Root-Knot Nematode, *Meloidogyne incognita*

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ABSTRACT

Root knot nematodes (*Meloidogyne* spp.) are among the most dangerous plant pathogens that attacked many hosts from all types of plants. The root-nematode species; *M. incognita* is the most widespread species all over the world. Copper (Cu) has been used a lot in various control treatments and in different forms as well. In this research, copper nanoparticles (CuNPs) were synthesized using two different methods (chemical and green-biological synthesis). Imaging's of NPs were done using a scanning electron microscope (SEM) to verify the size of the nanoparticles. The morphological images of chemically synthetized NPs (CCuNPs) showed many NPs size with an average of about 126 ɳm. However, the morphological images of CuNPs that prepared using green biosynthesis (GBCuNPs) showed particles with the average of about 85 ɳm. Three concentrations of 100, 150 and 200 ppm of either CCuNPs or GBCuNPs and the chemical nematicide, Nemaprop®10G (as a positive control) were tested, *in vitro*, against 2nd stage juveniles (J_2) of *M. incognita*. The results of mortality % of J_2 exposure to the tested treatments after 24 and 48 hours indicated that the CCuNPs treatments recorded the highest mortality % of J_2 after both 24 and 48 hours of exposure followed by that recorded with GBCuNPs treatments, while the lowest mortality % was recorded with Nemaprope®10G compared with the check treatment. According to the concentration main effect, the highest reduction percentages were recorded with the 100 and 200 ppm concentrations followed by that recorded with 150 ppm. The interaction between different treatments and its tested concentrations showed significant effective results compared to the check treatment.

Keywords: Nematicidal Activity; Chemical and Green Biosynthesis; SEM; Copper Nanoparticles; *Meloidogyne* spp.

INTRODUCTION

Root-knot nematodes (RKN) belonging to the genus *Meloidogyne* are important agricultural pests worldwide that cause extensive damage to a wide variety of economically important crops. Root-knot nematode species, *M. incognita* and *M. javanica* are considered the major species distributed worldwide and parasitized a large host range of economic crops. Otherwise, the occurrence of virulent isolates could break resistance on RKN-resistant plant cultivars. Disease complex caused by interactive effect of nematode and other soil organisms, e.g., fungi and bacteria resulted in significant reductions in absorbing water, mineral uptake, physiological and biochemical plant changes. Those modifications reduced plant host vigour and finally caused plant death (Masse *et al.*, 2002).

Decraemer and Hunt (2006) showed that there are more than 4100 species of nematode pathogens and they confirmed that the most dangerous and widespread is that belonged to the genus *Meloidogyne*. While, Jones *et al.* (2013) clarified that the root-knot nematodes occupy the first place among the 10 most dangerous phytonematode pathogens, which causes economic losses of about 77-80 billion dollars annually worldwide (Nicol *et al.*, 2011 and FAO, 2022). Global has also confirmed that sales of nematicides are on continuous increases, with a global record of 1.50 billion dollars in 2021, and in 2022 sales recorded of about 1.55 billion dollars. The expected sales volume will reach about 2.12 billion dollars in 2029 that indicates an increased awareness of the importance of controlling and reducing the damage caused by parasitic nematode (FAO, 2022).

Copper element, especially in the form of copper sulfate, has been used to resist many plant pathogens, especially plant-parasitic nematodes, singly or mixed with some organic compounds or traditional nematicides (Kim *et al.*, 2022).

Jehyeong *et al.* (2019) used copper sulfate mixed with organic acids as environmentally benign compounds against root-knot nematodes (RKN) infestation. Data indicated that the compound Mix-WP30 consisting of maleic acid and copper sulfate was efficient in reducing RKN infection by 51.72 % on tomato plants grown in a greenhouse compared to the chemical nematicide, Fosthiazate which resulted in 48.38 reduction %. The results indicated that copper has a significant role in combating parasitic nematodes especially RKN.

Engineered nanoparticles (NPs) (1–100 nm) that have demonstrated activity in suppressing plant diseases are metalloids, metallic oxides, nonmetals, and carbon

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nanomaterials. NPs have been integrated into disease management strategies as bactericides/fungicides and as nano fertilizers to enhance plant health. Although there are reports of over 18 different NPs of single element and carbon nanomaterials affecting diseases and/or plant pathogens. The elements, Ag, Cu, and Zn have received much attention so far. Some NPs act directly as antimicrobial agents while others their main functions were acting in altering the nutritional status of the host and thus activate their defense mechanisms. For example, AgNPs and CuNPs can be directly toxic to microorganisms. The other NPs of B, Mn, Si, and Zn appear to function in host defense as fertilizers. As demand for food production increases against a warming climate, nanoparticles will play an important role in mitigating the new challenges in disease management resulting in a reduction in active metals and other chemical inputs (Elmer *et al.*, 2018).

Nanotechnology impacts the horticulture efficiency with the assistance of the Nano-fertilizer, Nano-pesticides or Nano-herbicides which act as a smart delivery system to plants, likewise the different industries making definitions with Nanoparticles (100-250 nm) to improve their activities by expanding nanoparticles solubility in water (Javed *et al.*, 2019).

Mohamed *et al.* (2019) found that the continuous use of traditional nematicides has caused the emergence of resistance in some of them, which made it important to search for alternatives. They evaluated the efficacy of copper nanoparticles (CuNPs) *in vitro* against the root-knot nematode, *M. javanica*. The CuNPs were prepared by chemical methods and described using an electron microscope TEM and SEM. They found positive results from using CuNPs in some concentrations.

Gkanatsiou *et al.* (2019) synthesized CuNPs and FeNPs by biological methods and described using the electron microscope TEM and it was confirmed that it is in the required nanoscale and it was evaluated against *M. incognita* and *M. javanica* in tomato plants compared with using the chemical nematicide fosthiazate. Data indicated that CuNPs and FeNPs at lower concentrations resulted in a significant efficacy compared to fosthiazate.

While, Akhter *et al.* (2020) have successfully prepared CuNPs using the extract of the holophrastic plant, *Orobanche aegyptiaca*. The structural and morphological properties of CuNPs were determined by using X-ray Diffraction (XRD), Fourier-Transform Infrared Spectroscopy, Scanning Electron Microscopy, Energy Dispersive X-ray Spectroscopy (EDX) and Transmission Electron Microscopy (TEM) and they found terpene properties against root-knot nematode *M. incognita, in vitro*.

The goal of the current study was conducted to synthesize copper nanoparticles both chemically and green biosynthesized and to characterize its particles using scanning electron microscopy (SEM). The potential nematicidal activity of CuNPs prepared chemically as well as green biosynthesis against root-knot nematode, *M. incognita*, was investigated, *in vitro*, as another potential alternative for controlling such pathogens.

MATERIALS AND METHODS

I – Materials:

Source of nematicide:

The nematicide used in this study was Nemaprope® manufactured and kindly obtained by El-Helb Company for Pesticides and Agrochemicals. The active ingredient is Fosthizate 3-[butan-2-ylsulfanyl (ethyl) phosphoryl]-1,3- thiazolidin-2-one, molecular formula is $C_9H_8NO_3PS_2$. Rate of application is 12 kg/Fadden. Its toxicity is LD_{50} 73 mg/kg.

Chemicals used in the synthesis of nanoparticles:

Copper (II) nitrate trihydrate, linear formula is $Cu(NO_3)_2{\cdot}3\ H_2O$ and its molecular weight is 241.60.

II – Methods:

II-1- Preparation of plant extract:

The perennial herb plant, *Haplophyllum tuberculatum* (Forssk.)A.Juss, was used in this study for preparing biological green CuNPs. Whole plants of *H. tuberculatum* were collected from the northwest of Egypt at April 2021. Whole plants were thoroughly washed with distilled water and air-dried at room temperature for a week. Then it was placed in the oven at 40°C for a day. The dried WP was ground to a fine powder using a small laboratory mill. An amount of approximately 150 g of powdered materials of *H. tuberculatum* was extracted by placing it in 500 ml distilled water and kept in a dark bottle. Then, it was placed in a water bath at a temperature of 70° C with continuous stirring, for 3 hours. After that, it was left in a refrigerator at a temperature of 4° C and it was valid for use for a week (Raissi *et al.*, 2016 and Abdelkhalek *et al.*, 2020).

II-2- Chemical synthesis of copper nanoparticles (CCuNPs**):**

For preparation of chemical copper nanoparticles (CCuNPs), an amount of 0.1 M $Cu(NO_3)_2{\cdot}3\ H_2O$ was dissolved in 100 ml of distilled water, pH 4 using pH Meter (SELFCTR®-33K-2005-Spain). Slow drops of NaOH with a molarity of 0.1 M were added to the $Cu(NO_3)_2{\cdot}3H_2O$ solution with continuous shaking, without stopping until the pH reaches 14, till a light blue foamy precipitate was observed. After reaching pH 14, the solution left to precipitate for 24 hours without moving the bottle containing the solution. The precipitate was collected using a centrifuge at 11182 RCF for 30 minutes at 25˚C. Then, the precipitate was washed three times with distilled water and then the precipitate was washed three times with 90% ethanol. The precipitate was collected every washing time using a centrifuge at 6290 RCF for 15 minutes at the room temperature. Then the precipitate was placed in an oven for 16 hours at a temperature of 80 ˚C. The precipitate consisting the synthesized CuNPs is chemically ground in Krepsel before being placed in a Mavell oven (HOBERSAL® - HD-150- Spain) at 500 ˚C for 4 hours (Phiwdanga *et al.*,2013). The precipitate of CCuNPs is well ground using a mortar, then a weight of 0.24 g of powder were added to distilled water for preparing the desired concentrations then placed in an ultrasonic device (Sonication) (FALC®-DS-240DM-10L) for 72 hours at a temperature of 70˚C.

II-3- Preparation of green synthesis copper nanoparticles:

An amount of 0.2 M aqueous $Cu(NO3)_2 \cdot 3H_2O$ solution was prepared and stored in brown bottle, then mixed with 400 ml of the plant *H. tuberculatum* extract. Then, we add 2 M of $Cu(NO3)_2 \cdot 3\ H_2O$ very slowly with continuous stirring using a magnetic stirrer (SELFCTR® -AGIMATIC-ED-Spain) at 500 rpm at the room temperature. The precipitate was collected using a centrifuge (HERMLE®-Z36HK-302.00V01-Germany) at 8561 RCF for 30 minutes at 15˚C. Then the precipitate was washed twice with distilled water, then the precipitate washed twice with ethanol 90%, and the precipitate was collected after each washing time using a centrifuge at 1572 RCF for 15 minutes. Then the precipitate was placed in an oven for 18 hours at a temperature of 50˚C (Murthy *et al.,* 2020). The precipitate of GBCuNPs was well ground using a mortar, then a weight of 0.24 g of powder was added to distilled water for preparing the desired concentrations then placed in an ultrasonic device (Sonication) (FALC®-DS-240DM-10L) for 20 hours at a temperature of 70 ˚C.

II-4- Characterization of copper nanoparticles:

Characterization of copper nanoparticles were done using a scanning electron microscopy (SEM) in the laboratory of Egyptian Japanese University, New Borg El Arab, Alexandria.

Scanning electron microscopy (SEM), Model (JEOL-JSM-6010LV-Japan), was used as a method for high-resolution imaging of NPs surfaces. SEM analysis of images taken was done. Samples of CuNPs were prepared for SEM characterization by adding dried CuNPs on the SEM adhesive paper (0.5 × 0.5 cm). CuNPs glued on the adhesive paper were photographed and samples were placed in the SEM device to make them more electron passive.

II-5- Root-knot nematodes inoculum preparation:

The root-knot nematode *M. incognita* Kofoid and White (Chitwood) were obtained from laboratory of Plant Nematology, Department of Plant Pathology, Faculty of Agriculture, Alexandria University. The root-knot nematode eggs and 2*nd* stage juveniles (**J_2**) were extracted from the infected tomato roots using sodium hypochlorite (NaOCl) solution (Hussey and Barker, 1973). Root-knot nematode eggs and the hatched **J_2** were placed in sterile distilled water and used in all tests.

II-6- Effect of chemical synthesis nanoparticles (CCuNPs), green synthesis nanoparticles (GBCuNPs) and the nematicide, Nemaprope®10G on J_2 mortality % of root-knot nematode, *M. incognita* under laboratory condition.

The effect of three doses of 100,150 and 200 ppm of chemical synthesis nanoparticles (CCuNPs), green synthesis nanoparticles (GBCuNPs), and Nemaprope®10G on J_2 mortality were tested under laboratory condition. Treatments were done using 24-well plates (Corning); each well received 2 ml of each treatment. A total of 120 juveniles of *M. incognita* (J_2), suspended in 15 ml water, was added in each well as described by Ntalli *et al.* (2016). NPs treatments were applied in three doses of 100,150 and 200 ppm dissolved in distilled water. Also, three doses of 100,150 and 200 ppm of Nemaprope® were prepared. Each treatment was repeated three times. Root-knot nematode J_2, added only in sterile distilled water without NPs treatments, were used to serve as a control treatment. Treatments were maintained at 25±2 ˚C in an incubator. Observations were recorded after 24 and 48 hours of adding J_2 in each treatment.

RESULTS

The morphological images of chemically synthesized Cu-nanoparticles which prepared with using $Cu(NO_3)_2.3H_2O$ was illustrated in Fig. 1. It is clear that there are very many particles in the required nano size, which were (10-150 ɳm) diameter, and it is clear that the average was about 126 ɳm. Likewise, Fig. (2) showed morphological images of CuNPs that prepared using green biosynthesis. Similarly to the Fig. (1), the image of Fig. (2) showed many NPs in the required nano size and it was clear, from the measurements taken under the SEM, that the average of NPs was about 85 ɳm.

Data in Table (1) showed the effect of chemically and biologically synthesized copper nanocomposites and the chemical nematicide, Nemaprop®10G on percentage numbers of dead root-knot nematode, *M.*

incognita juveniles (J_2) after 24 hours of the tested treatments (Fig. 3). Results of treatment main effect indicated that chemical synthesis nanoparticles (CCuNPs) recorded the highest mortality % (73.4 %) of *M. incognita* juveniles followed by that of green synthesis nanoparticles (GCuNPs) with mortality of 63.59 %. The lowest mortality % of 6.14 % was recorded with Nemaprope®10G compared with the check treatment (control treatment).

According to the concentration main effect, the highest reduction % of 50.29 and 47.41 % were noted with 100 and 200 ppm concentration records, respectively followed by 150 ppm concentration with reduction of 44.51 %.

In relation to the interaction between CuNPs treatments and concentrations, data showed that the highest reduction record (90 %) was obtained with [100 ppm of CCuNPs] followed by that of [150 and 200 ppm NPs concentrations of CCuNPs and GBCuNPs] with 60.17-70.90 reduction %.

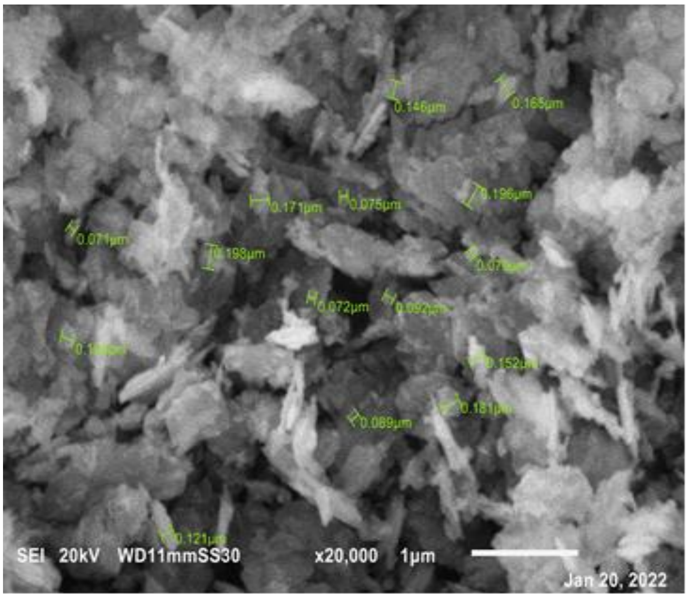


Fig. 1. Scanning electron microscopy (SEM) images of chemically synthesis Cu nanoparticles (CCuNPs)

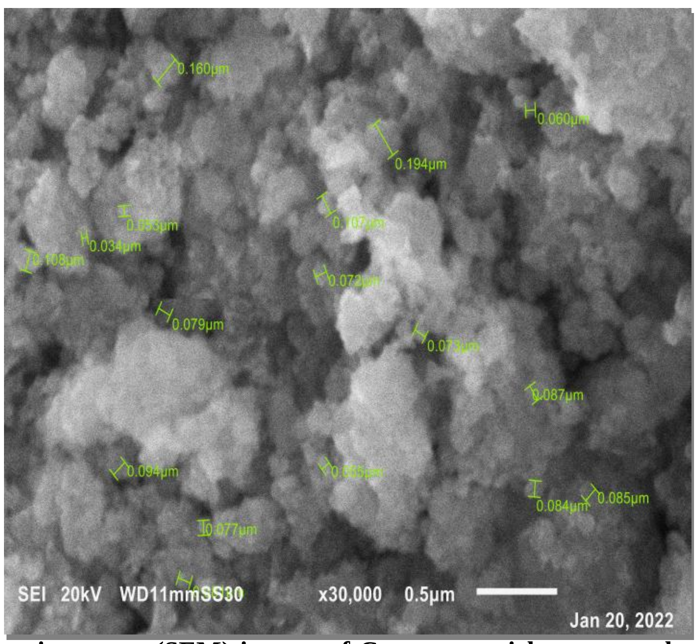


Fig. 2. Scanning electron microscopy (SEM) images of Cu nanoparticles prepared using green Bio-Synthesis method

The least reduction records were noticed with treatments of Nemaprop®10G which were mostly similar to that obtained with the check treatment at the all the tested concentrations with reductions % ranged between 0-16.2 reduction %.

Table (2) and Fig. (4) showed the results of chemically (CCuNPs) and green biologically (GBCuNPs) synthesized copper nanocomposites and the nematicide, Nemaprop®10G on percentage numbers of dead root-knot nematode, *M. incognita* juveniles after 48 hours' treatment.

According to the treatment main effect, data showed that CCuNPs recorded the same results previously obtained at 24 hours. The highest J_2 mortality % (91.15%) of *M. incognita* was obtained with CCuNPs treatment followed by that recorded with treatment of GBCuNPs with 82.62 reduction %. The lowest mortality % was recorded with Nemaprop® 10G with 8.29 % compared with results of the check treatment (control treatment).

Regarding to the concentration main effect, the highest reductions % of 64.21 and 59.20 reduction % were recorded with the concentrations of 200 and 100 ppm, respectively followed by 150 ppm concentration with reduction of 56.82 %.

In relation to the interaction between CuNPs treatments and concentrations, data showed that the highest reduction record (100 %) was obtained with [200 ppm concentration of CCuNPs] followed by that of [100 ppm of CCuNPs] and [150 and 200 ppm concentrations of GBCuNPs] with 85.23- 90.90 reduction %. The least reduction records were detected with treatments of Nemaprop®10G which were nearly similar to that of obtained with the check treatment at the all tested concentrations with reductions ranged between 0-20.94 %.

Table 1. Effect of chemically (CCuNPs) and green biologically (GBCuNPs) synthesized copper nanocomposites and the nematicide, Nemaprop 10G on percentage numbers of dead root-knot nematode, *M. incognita* juveniles, 24 hours after treatments

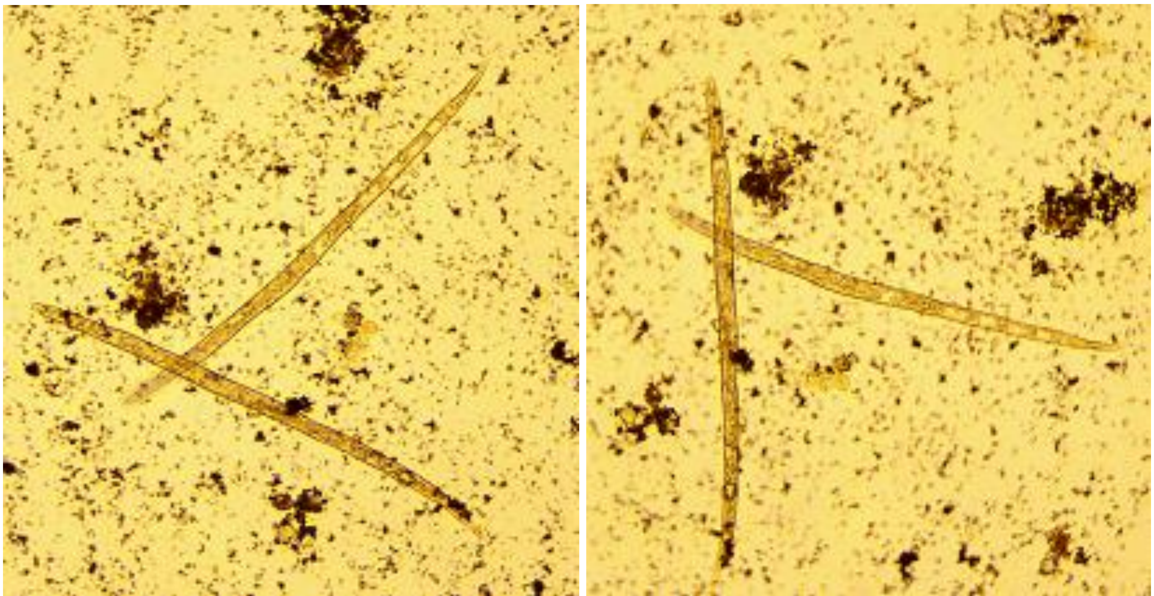
Treatment	Concentration (ppm)			Treatment main effect
	100	**150**	**200**	
Check, MI alone	0.00^x g	0.00 g	0.00 g	**0.00 D**
CCuNPs + MI	90.00 a	68.94 bcd	60.17 de	**73.04 A**
GBCuNPs + MI	53.90 e	65.97 cd	70.90 bc	**63.59 B**
Nemaprop 10G + MI	0.00 g	2.22 g	16.20 f	**6.14 C**
Concentration main effect	**50.29 A**	**44.51 B**	**47.41 A**	

^{x}=percentage of dead juveniles, data are average of 3 replicates. Data with the same capital letter(s) of treatment main effects, in the last column, and of concentration main effect (row) are not significantly different at p=0.05. Interaction data with the same small letter(s) are not significantly different at p=0.05.

Table 2. Effect of chemically (CCuNPs) and green biologically (GBCuNPs) synthesized copper nanocomposites and the nematicide, Nemaprop 10G on percentage numbers of dead root-knot nematode, *M. incognita* juveniles, 48 hours after treatments

Treatment	Concentration (ppm)			Treatment main effect
	100	**150**	**200**	
Control, MI alone	0.00 h	0.00 h	0.00 h	**0.00 D**
CCuNPs + MI	90.90 bc	82.55 de	100.0 a	**91.15 A**
GBCuNPs + MI	78.81 e	84.44 bce	85.23 bcde	**82.62 B**
Nemaprop 10G + MI	0.0 h	3.92 g	20.94 f	**8.29 C**
Concentration main effect	**59.20 B**	**56.82 C**	**64.21 A**	

^{x}=percentage of dead juveniles, data are average of 3 replicates. Data with the same capital letter(s) of treatment main effects, in the last column, and of concentration main effect (row) are not significantly different at p=0.05. Interaction data with the same small letter(s) are not significantly different at p=0.05



a b

Fig. 3. Dead *M. incognita* J2 after 24 (a) and 48 (b) hours treatment with chemically synthesized copper nanoparticles (CCuNPs)

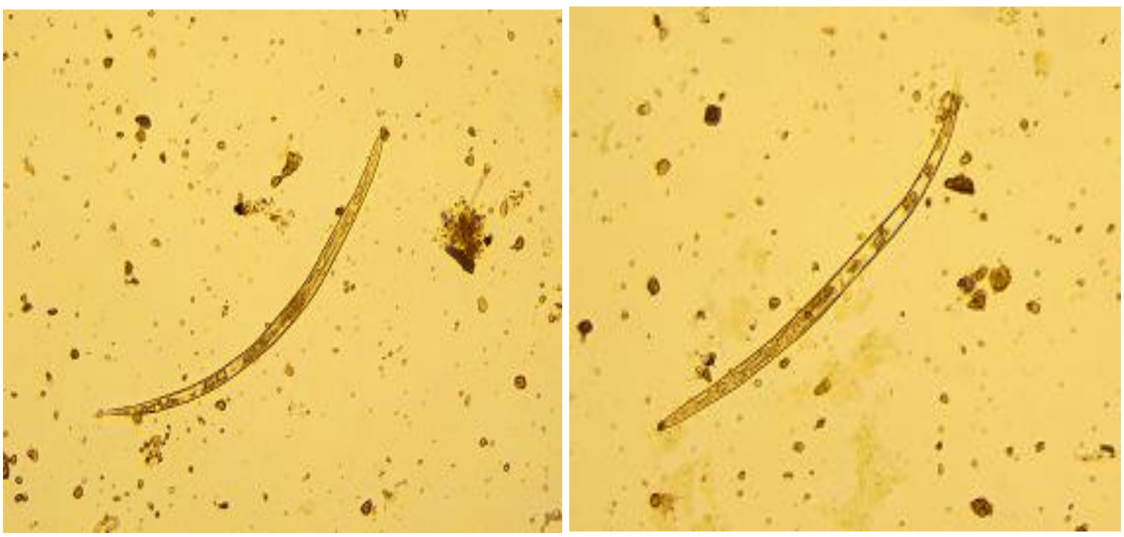


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Fig. 4. Dead *M. incognita* J2 after 24 (a) and 48 (b) hours treatment with green biosynthesized copper nanoparticles (GBCuNPs)

DISCUSSION

Root knot nematode, *Meloidogyne incognita* is the most widespread species all over the world. Masse *et al.* (2002) reported that *M. incognita* and *M. javanica* are considered the major species distributed worldwide and parasitized a wide host range of economic crops. Otherwise, the occurrence of virulent isolates could break resistance on RKN-resistant plant cultivars. However, disease complex caused by the interactive effects of parasitic nematodes and the other soil organisms, e.g., fungi and bacteria resulted in significant reductions in absorbing water, mineral uptake, physiological and biochemical plant changes. As well as, reduced plant host vigour and finally caused plant death.

Copper element, especially in the form of copper sulfate, has been used to resist many plant pathogens, especially plant-parasitic nematodes, singly or mixed with some organic compounds or traditional nematicides (Kim *et al.,* 2022). Copper nanoparticles (CuNPs) were synthesized using two different methods; chemical (CCuNPs) and green-biological (GBCuNPs) synthesis. Images of NPs obtained were photographed using a scanning electron microscope (SEM) and showed that the size of NPs prepared by chemical and green biosynthesis were about 126 and 85 ɳm, respectively.

Mohamed *et al.*, (2019) evaluated the efficacy of copper nanoparticles (CuNPs) *in vitro* against *M. javanica*. The CuNPs were prepared by chemical methods and described using an electron microscope TEM, SEM. They found positive results from using CuNPs in some concentrations. Gkanatsiou *et al.* (2019) synthesized CuNPs, FeNPs by biological methods and described using the electron microscope TEM and it was confirmed that it is in the required nanoscale and it was evaluated against *M. incognita* and *M. javanica* nematodes in tomato plants compared with using the chemical nematicide fosthiazate. Data indicated that CuNPs and FeNPs at lower concentrations resulted in a significant efficacy compared to fosthiazate. Also, Akhter *et al.* (2020) have successfully prepared CuNPs using the extract of the holophrastic plant, *Orobanche aegyptiaca*. The structural and morphological properties of CuNPs were determined by using X-ray Diffraction (XRD), Fourier-Transform Infrared Spectroscopy, Scanning Electron Microscopy, Energy Dispersive X-ray Spectroscopy (EDX) and Transmission Electron Microscopy (TEM) and they found terpene properties against root-knot nematode *M. incognita, in vitro*.

Jehyeong *et al.* (2019) used copper sulfate mixed with organic acids as environmentally benign compounds against root-knot nematodes (RKN) infestation. Data indicated that the compound Mix-WP30 consisting of maleic acid and copper sulfate was efficient in reducing RKN infection by 51.72 % on tomato plants grown in a greenhouse compared to the chemical nematicide, Fosthiazate which resulted in 48.38 reduction %. The results indicate that copper has a significant role in combating parasitic nematodes.

Nanoparticles have been integrated into disease management strategies as bactericides/fungicides and as nano fertilizers to enhance plant health. Although there are reports of over 18 different NPs of single element and carbon nanomaterials affecting plant pathogens. Elmer *et al.* (2018) found that the engineered NPs of 1–100 nm diameter demonstrated activity in suppressing plant disease pathogens. Some NPs act directly as antimicrobial agents while others their main functions were acting in altering the nutritional status of the host and thus activate their defense mechanisms. They reported that AgNPs and CuNPs can be directly toxic to microorganisms. The other NPs of B, Mn, Si, and Zn appear to function in host defense as fertilizers. As demand for food production increases against a warming climate, nanoparticles will play an important role in mitigating the new challenges in disease management resulting in a reduction in active metals and other chemical inputs. Similarly, Javed *et al.* (2019) reported that nanotechnology impacts the horticulture efficiency with the assistance of the nano-fertilizer, nano-pesticides or nano-herbicides which act as a smart delivery system to plants, likewise the different industries making definitions with Nanoparticles (100-250 nm) to improve their activities by expanding nanoparticles solubility in water.

Results of mortality % of J_2 exposure to the tested treatments; CCuNPs or GBCuNPs and the chemical nematicide, Nemaprop®10G for 24 and 48 hours indicated that the CCuNPs treatment recorded the highest mortality % of J_2 after both exposure times (73.04 and 91.15 reduction %, respectively) followed by that recorded with GBCuNPs treatment with 63.59 and 82.62 reduction %, respectively while the lowest mortality % of 6.14 and 8.29 reduction % were recorded with Nemaprope®10G compared with the check treatment. According to the concentration main effect, the highest reductions percentages were recorded with the 100 and 200 ppm concentrations followed by that recorded with 150 ppm. The interaction between different treatments and its tested concentrations showed significant results compared to the check treatment.

From the above, it is a clear difference between the average sizes in the NPs diameter between that formed from either the two methods, chemically or biologically synthesis. This could be explaining the differences between the results of J_2 mortality of *M. incognita* treated with NPs tested in the laboratory because all the other factors are constant; age of J_2 collected, time of application, treatment concentration and temperature. The only difference in the CuNPs synthesis process, chemical and green bio-synthesis, was the diameters of CuNPs resulted, 126 ɳm and 85 ɳm for chemical or green biosynthesis, respectively.

Conclusively, it can be concluded that the CuNPs may provide an alternative nematicide against the root-knot nematode, *M. incognita*. Further studies are needed in order to determine the optimum doses and application methods to obtain best nematicidal activity without considerable hazards.

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REFERENCES

Abdelkhalek, A., M. Z. M.Salem, E. Hafez, S. I. Behiry and S. H. Qari. 2020.The Phytochemical, antifungal, and first report of the antiviral properties of Egyptian *Haplophyllum tuberculatum* extract. Biology, (9): 248.

Akhter, G., A. Khan, S.G. Ali, T. A. Khan, K.S. Siddiqi and H.M. Khan. 2020.Antibacterial and nematicidal properties of biosynthesized Cu nanoparticles using extract of holoparasitic plant. SN Applied Sciences 2: 1268 (Short Communication). *https://doi.org/10.1007/s42452-020-3068-6*.

Decraemer, W. and D. J. Hunt. 2006. Structure and classification. In: Plant Nematology (Perry, R.N. and Moens, M. eds), pp. 3–32. Wallingford, Oxfordshire, CAB International.

Elmer, W., C. Ma and J. White. 2018. Nanoparticles for plant disease management. Science Direct Current Opinion in Environmental Science and Health 66: 66-70.

FAO.2022. Crop Protection/Nematicides Market. *https://www.fortunebusiness insights.com/industry-reports/nematicides-market-101609*.

Gkanatsiou, Ch., N. Ntalli, U. Menkissoglu-Spiroudi, C. Dendrinou-Samara. 2019. Essential Metal-Based Nanoparticles (Copper/Iron NPs) as potent nematicidal agents against *Meloidogyne* spp. J. Nanotechnol. Res. 1 (2): 44-58.

Hussey, R. S. and K. R. Barker.1973. Comparison of methods of collecting inocula of *Meloidogyne* spp., including a new technique. Plant Disease Reporter, 59: 1025–1028.

Javed, Z., K. Dashora, M. Mishra, Fasake, D. Vinayak and A. Srivastva. 2019. Effect of accumulation of nanoparticles in soil health- concern on future. Front Nanosci Nanotech 5: 1-9. *doi: 10.15761/FNN. 1000181*.

Jehyeong, Y.A., P. Ran, J.K.H. Yoon, S.N. Jeong, Y.S.H. Hee and P.J. Woong. 2019. Control of root-knot nematodes by a mixture of maleic acid and copper sulfate. Applied Soil Ecology, 141: 61-68.

Jones, J.T., A. Haegeman, E.G.J. Danchin, H.S. Gaur, J. Helder, M.G.K. Jones, T. Kikuchi, G. K. Michael, T. Kikuchi, Manzanilla-Lopez, Rosa, Palomares-Rius, Juane, W. M. L. Wesemael and R.N. Perry. 2013. Top 10 plant-parasitic nematodes in molecular plant pathology, Molecular Plant Pathology 14(9): 946–961.

Kim, S., H. M. Kim, H. J. Seo, J. Yeon, A. R. Park, Nan H. Yu, S. Jeong, J. Y.Chang, J. Kim and H. W. Park. 2022. Root-knot nematode (*Meloidogyne incognita*) control using a combination of *Lactiplantibacillus plantarum* WiKim0090 and copper sulfate. Microbiol Biotechnol. 28 (8): 960-966. *doi: 10.4014/jmb.2205.05019*.

Masse, D., E. Pate, N. Ndiaye-Faye and P. Cadet. 2002. Effect of fallow improvement on the nematode community in the Sudanian region of Senegal. European Journal of Soil Biology 38: 205-211.

Mohamed, E. A., S. F. Elsharabasy and D. Abdulsamad. 2019. Evaluation of *in vitro* nematicidal efficiency of copper nanoparticles against root-knot nematode *Meloidogyne incognita*. South Asian Journal of Parasitology 2(1): 1-6.

Murthy, A. H.C., T. Desalegn, M. Kassa, B. Abebe and T. Assefa. 2020.Synthesis of green copper nanoparticles using medicinal plant *Hagenia abyssinica* (Brace) JF. Gmel. leaf extract: antimicrobial properties. Journal of Nanomaterials, Article ID 3924081. 12 p.

Nicol, J. M., S. J. Turner, D. L. Coyne, L. den Nijs, S. Hockland and Z. T. Maafi. 2011. Current nematode threats to world agriculture. Edited by: Jones, J; Gheysen, G; Fenoll, C. (provided by Clarivate) edit. In "Genomics and Molecular Genetics of Plant-Nematode Interactions" :21-43. *doi10.1007/978-94-007-0434-3_2*.

Ntalli, N.G., Ch. Oplos and M. Michailidis. 2016. Strong synergistic activity and egg hatch inhibition by (E,E)-2,4-decadienal and (E)-2-decenal in Meloidogyne species. J. Pest Sci. 89:565-579.

Phiwdanga, K., S. Suphankij, W. Mekprasarta and W. Pecharapa. 2013. Synthesis of CuO nanoparticles by precipitation method using different precursors. Science Direct Energy Procedia 34: 740–745.

Raissi, A., M. Arbabi, J. Roustakhiz, M. Hosseini. 2016. ***Haplophyllum tuberculatum*** **an overview.** J. Herb. Med. Pharmacol. 5(4): 125-130.

الملخص العربي

التأثير الإبادى لمركبات النحاس النانونية المخلقة كيماوياً وبيولوجياً ضد نيماتودا تعقد الجذور *Meloidogyne incognita*

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تعتبر نيماتودا تعقد الجذور .*Meloidogyne* spp من أخطر مسببات الأمراض النباتية التي تهاجم العديد من العوائل النباتية. ويعتبر النوع *M. incognita* الأكثر انتشارًا في جميع أنحاء العالم . وقد تم استخدام النحاس (Cu) فى مقاومة الكثير من مسببات الأمراض النباتية وبأشكال مختلفة أيضًا. وفي هذا البحث تم تخليق دقائق النحاس النانوية (CuNPs)باستخدام طريقتين مختلفتين (التخليق الكيميائي (CCuNPs) والحيوى (GBCuNPs)) وتم تصوير الدقائق الناتجة باستخدام المجهر الإلكتروني الماسح (SEM) للتحقق من حجم الدقائق النانوية. أظهرت الصور الظاهرية (المورفولوجية) للدقائق المُصنَّعة كيميائياً (CCuNPs) العديد من الأحجام النانونية بمتوسط حوالي ١٢٦ نانوميتر. وكذلك أظهرت الصور الظاهرية للدقائق النانونية والتي تم تحضيرها باستخدام التخليق الحيوي (GBCuNPs) أن متوسطها حوالي ٨٥ نانوميتر. تم اختبار ثلاثة تركيزات ١٠٠ ، ١٥٠ ، ٢٠٠ جزء في المليون من دقائق النانو CCuNPs ، GBCuNPs ، والمبيد النيماتودى الكيميائي نيمابروب ١٠% (Nemaprop®10G)في المعمل (*in vitro*) ضد يرقات الطور الثانى النيماتودية (J2) للنوع (*M. incognita*). وأخذت نتائج معدل نسبة اليرقات الميتة (% mortality) نتيجة تعرض يرقات النيماتودا J2 للمعاملات المختبرة بعد ٢٤ و ٤٨ ساعة من المعاملة . وبينت النتائج أن المعاملة بدقائق النحاس النانوية المخلقة كيماوياً (CCuNPs) سجلت أعلى نسبة موت لليرقات المعاملة لمدة ٢٤ و ٤٨ ساعة من التعرض ، تلتها المعاملة بدقائق النانو (GBCuNPs) ، في حين سجلت أقل نسبة موت لليرقات فى المعاملة بالمبيد النيماتودى نيمابروب ١٠% مقارنةً بمعاملة المقارنة (الكنترول). ووفقًا لتأثير التركيز الرئيسي (concentration main effect) فقد سجلت أعلى نسبة تخفيض معنوى لموت اليرقات مع التركيزين ١٠٠ و ٢٠٠ جزء في المليون تلى ذلك نسبة الموت المسجلة بالتركيز ١٥٠ جزء في المليون. وأظهر التفاعل بين المعاملات المختلفة وتركيزاتها المختبرة وجود فروق معنوية مقارنة بمعاملة المقارنة.