Original Research Article

Evaluation of *In Vitro* Nematicidal Efficiency of Copper Nanoparticles Against Root-Knot Nematode *Meloidogyne incognita*

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ABSTRACT

Root-knot nematodes (*Meloidogyne* **spp.) are considered among the most deteriorating soilborne parasites that can significantly affect many plants. These nematodes are developing increased resistance against nematicides used currently to control them, therefore, continued use of these nematicides poses a challenge, thereby giving rise to the need for newer alternatives.** This paper evaluated the *In vitro* nematicidal efficiency of copper nanoparticles (CuNPs) against root-knot nematode, *Meloidogyne incognita*. In this study, CuNPs were prepared according to the chemical reduction method; physicochemical characterization of CuNPs was done using UV-Vis spectroscopy, Dynamic Light Scattering and Transmission Electron Microscopy. When second stage juveniles (J2) of *M. incognita* were incubated in soil saturated with CuNPs (spherical shape; 100 nm diameter) for 3 days, it was found that J2 mortality is directly proportional to the concentration of CuNPs and 0.2 g/L was sufficient to cause 100% mortality. Statistical analysis showed that all mortalities caused by treatment with CuNPs at different concentrations were statistically significant compared with non-treated control. Conclusively, this paper may provide a potential alternative nematicide against root-knot nematode *Meloidogyne incognita*. Further *In vivo* and toxicological research on CuNPs should be conducted in order to assess the possible applicability of such nanoparticles as a nematicide.

***Keywords*:** *Meloidogyne incognita*; Nematicide; Copper Nanoparticles; Nematicide alternative.

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1. INTRODUCTION

Meloidogyne spp. was first reported in cassava (*Manihot esculenta*) by Neal in 1889 [1]; Since then, root-knot nematodes (*Meloidogyne* spp.) are considered among the most deteriorating soilborne parasites that can significantly affect many field crops, trees and turfgrass [2]. Nematodes are characterized with a broad host range of greater than 3,000 plant species [3]. Furthermore, it was reported that around 5% of the world crop production was lost annually due to infection with *Meloidogyne* species [4] and the losses can reach up to 64% of the yield [5-7].

Negative effects of nematode infections are not limited to decreased productivity of the economical crops, since it can also affect the playability and aesthetic quality of golf courses [8].

Meloidogyne species encompass 98 species, among them *M. incognita, M. javanica, M. hapla,* and *M. arenaria* are considered the most common [9].

After banning Nemacur in 2008 due to environmental concerns, there is a dire need for developing newer efficient alternatives to control such plant-parasitic nematodes. In this respect, the narrow range effectiveness characterizing biological control agents limits its applicability. For example, the bacterial parasite, *Pasteuria* sp. can control sting nematodes (*Belonolaimus longicaudatus*) [10]; however, it cannot affect the other species of plant-parasitic nematodes such as root-knot nematodes (*Meloidogyne* spp.).

Nanotechnology is considered a promising and effective means for controlling root-knot nematode, wherein some papers reported the nematicidal effect of silver nanoparticles (AgNPs) [11, 12], gold nanoparticles [26] and silicon carbide nanoparticles [24] against root-knot nematodes. By virtue of the well-established namaticidal effect of AgNPs, AgNPs were proposed [13] as a potential alternative nematicide.

In this regard, many papers have established a robust emphasis on the antimicrobial effect of CuNPs [14-16]; thus, in this paper, we evaluate the *In vitro* nematicidal efficiency of CuNPs against J2 *M. incognita* as another potential alternative for controlling such parasites.

2. MATERIALS AND METHODS

A- Chemicals

All chemicals used were analytical grade of purity and were used without further purification.

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L-ascorbic acid (Future Modern Co., Egypt.); Cetyl trimethylammonium bromide (CTAB) (Sigma-Aldrich, Egypt.); copper sulfate pentahydrate (Elnasr Pharmaceuticals Co., Egypt) were used to prepare CuNPs.

B- Methods

Preparation of CuNPs

CuNPs were prepared according to the chemical reduction method [17]. In this method, L-ascorbic acid was used as a reducing agent, in the presence of CTAB as a cationic surfactant, to reduce copper cations provided from copper sulfate pentahydrate into copper atoms, which were aggregated and developed into copper nanoparticles, with their characteristic reddish brown color, at pH of 6.8 and temperature of 85^{o}C. CuNPs were centrifugally (4000 rpm) collected for further characterization and application.

Characterization of CuNPs

The characteristic surface plasmon resonance of the synthesized CuNPs was detected using UV-Vis Spectrophotometer (ORION AQUAMATE 8000). Also, particles size distribution by number of CuNPs was detected using Dynamic light scattering (DLS) (Zetasizer nano series (Nano ZS), Malvern, UK). Moreover, the shape of the CuNPs was detected through Transmission Electron Microscopy (Tecnai G20, Super twin, double tilt, FEI, Netherland).

***In vitro* application of CuNPs**

21 jars (300 cm^3) were filled with soil composed of 1:1 beet moss and sand. Water saturation level of 300 cm^3 soil was determined to be 100 ml. Each filled jar was inoculated with 1,000 larva second stage juveniles (J2) and homogenized well. Then, each jar was saturated with 100 ml of copper nanoparticles solution at different concentrations, (0.02, 0.04, 0.06, 0.08, 0.1 and 0.2 g/L). Soil jars saturated with water were used as a control. Each concentration was applied in triplicate. All jars were incubated at room temperature for 3 days. After the mentioned exposure time, nematodes were extracted, counted and mortality was calculated according to equation (1).

$$\text{Mortality (\%)} = \left(\frac{Number\ of\ Dead\ Nematodes}{Total\ Number\ of\ Nematodes}\right) \times 100 \qquad (1)$$

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Statistical analysis

SPSS 22 software (Chi Square Method) was used at $P \leq 0.05$ to distinguish between the nematicidal efficacies. Each treatment was conducted in triplicate, and the whole experiment was repeated twice [18].

3- RESULTS

Physicochemical Characterization of CuNPs

Successful synthesis of CuNPs was confirmed through exhibiting their characteristic surface plasmon resonance peak which was detected using UV-Vis Spectrophotometer (ORION AQUAMATE 8000) at wavelength of 572 nm [27], as shown in Figure (1).

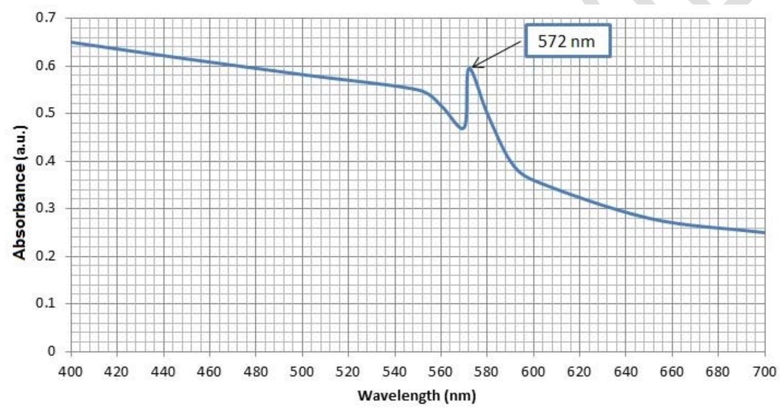

Figure (1): characteristic surface plasmon resonance peak of CuNPs at 572 nm.

Also, Dynamic Light Scattering revealed that the average size of the synthesized CuNPs was about 100 nm; as shown in Figure (2).

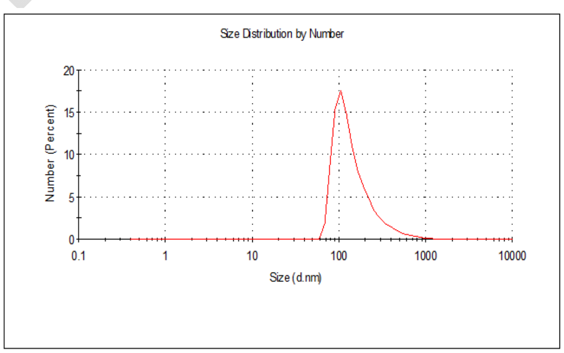

Figure (2): Particle size distribution by number of CuNPs, showing the average particle size of about 100 nm.

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In addition, Transmission Electron Microscopy revealed that the synthesized CuNPs have spherical shape, as shown in Figure (3).

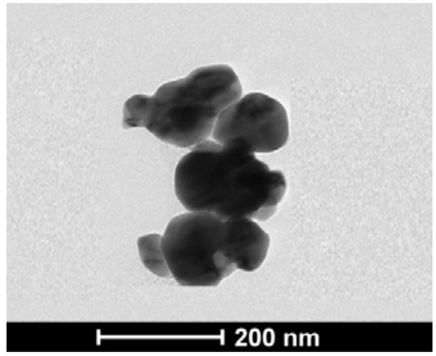

Figure (3): Transmission Electron Micrograph of the synthesized CuNPs showing the spherical shape of the particles.

Evaluation of the nematicidal effect of CuNPs

Statistical analysis showed that all concentrations of CuNPs exhibited significant inhibitions of the J2 *M. incognita*. It was shown that CuNPs have a linear nematicidal effect against J2 *M. incognita*, i.e. the higher the concentration of CuNPs, the higher the mortality of nematodes. The concentration of 0.2 g/l was sufficient to completely inactivate all nematodes. Viable nematodes are circular or curved, while dead nematodes are straight, as shown in Figure (4).

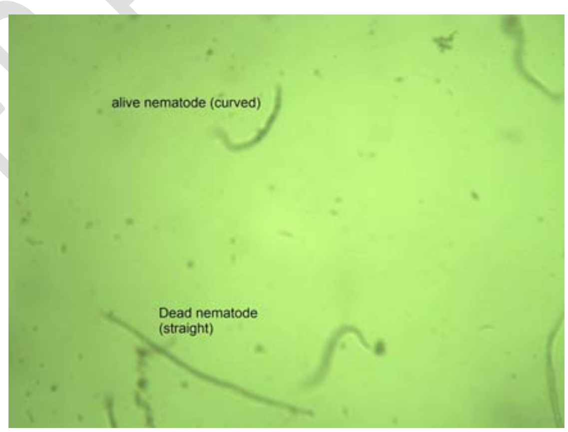

Figure (4): the shape of viable vs. dead nematodes under compound microscope.

Concentration-dependent mortality of *M. incognita* caused by CuNPs can be shown in Figure (5).

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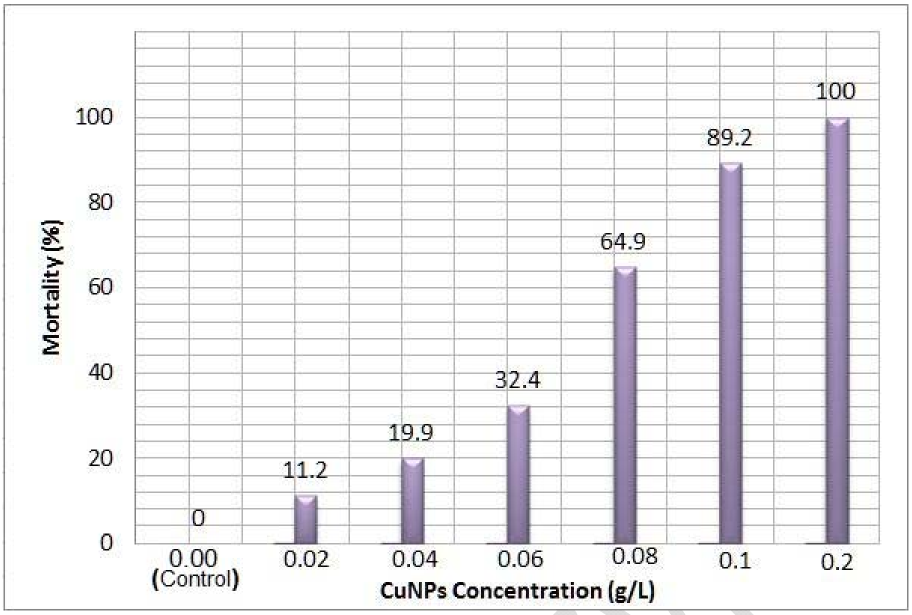

Figure (5): a graph shows the direct proportionality between CuNPs concentration and J2 mortality.

4- DISCUSSION

This study has emphasized on the potential *In vitro* nematicidal effect exhibited by CuNPs against the second stage juveniles (J2) of root-knot nematodes, *M. incognita*; this was demonstrated through the significant increase of J2 mortality at various concentrations of CuNPs compared with non-treated control.

Recently, the effect of silicon carbide nanoparticles on hatching and survival of *M. incogneta* was investigated [24]. In that study, it was found that silicon carbide nanoparticles neither affect hatchability of larvae nor survival of second stage juveniles (J2) of *M. incogneta*. Which urge the need for assessing the nematicidal effect of more toxic nanoparticles against such tolerant nematodes. In this regard, CuNPs may offer that alternative due to their potential nematicidal effect against *M. incogneta*, as shown from the present study.

Also, among different types of nanoparticles, the nematicidal effect of AgNPs has extensively studies; but, from this investigation, it is noteworthy that CuNPs could exhibit a significantly higher nematicidal effect than AgNPs at the same concentration against J2 of root-knot nematodes, *M. incogneta*. In this regard, it was reported that 200 ppm of AgNPs could cause a mortality of 52% at the third day of direct exposure in water [19]. On the other hand, CuNPs at the same concentration

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could achieve a mortality of 100% after 3 days of indirect exposure in soil. This may due to the profound toxic effect of copper nanoparticles causing DNA damage, which is in contrasts to the more mild effect of AgNPs, which depends mainly on disturbance of many cellular mechanisms such as synthesis of ATP, permeability of the cellular membrane and response to the oxidative stresses in prokaryotes [20, 21] and eukaryotes [12, 22].

In addition, it was reported [23] that the highest percentage of mortality achieved after 3 days of direct exposure of second stage juveniles (J2) to AgNPs was 95%; while higher mortality percentage (100%) was attained using CuNPs, despite the indirect exposure, which reflect the superior nematicidal efficiency of CuNPs over both silicon carbide nanoparticlesand AgNPs. .

Furthermore, the non-specific nematicidal effect of copper nanoparticles provided a relative advantage over the microbial agents of bio-control, which are limited with their relatively high specific host range among different nematode species.

But some concerns may arise due to the emphasized toxicity of CuNPs [25]. In this regard, our paper just confirms the nematicidal effect of copper nanoparticles, this effect can be exploited to control nematodes infecting, for example, ornamental plants in pots or turfgrass, but not to control nematodes infecting, for example, edible crops; so as not to harm the human or environment. Otherwise, further research should be conducted to minimize such toxic effect of CuNPs through, for example, masking CuNPs or loading them on **non-toxic matrix such that increase its specific targeting to only nematodes**.

5- CONCLUSION

To sum up all, it can be concluded that CuNPs may provide an alternative nematicide against the root-knot nematodes, *M. incogneta*. But, further research should be conducted in order to investigate the environmental consequences of CuNPs, hence determining the optimum doses and methods that can be applied in field without considerable hazards.

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