Original Research Article

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

ABSTRACT

Root-knot nematodes (*Meloidogyne* spp.) are considered among the most deteriorating soilborn parasites that can significantly affect many field crops; current nematicides face a challenge in terms of resisting them and their environmental consequence, thus the need for new alternatives arised. This study evaluated the *In vitro* nematicidal efficiency of copper nanoparticles against root-knot nematode, *Meloidogyne incognita*. Copper nanoparticles were prepared according chemical reduction method, and characterized using Uv-vis spectroscopy, DLS and TEM. When second stage juveniles (J2) of *M. incognita* were exposed to Copper nanoparticles in soil saturated with Copper nanoparticles (100 nm) suspensions at 0.02, 0.04, 0.06, 0.08, 0.1 and 0.2 g/L for 3 days, J2 mortalities were 11.2, 19.9, 32.4, 64.9, 89.2 and 100%, respectively.

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

INTRODUCTION

Meloidogyne spp. was first reported in cassava (*Manihot esculenta*) by Neal in (1889). (Neal, 1889). Since then, Root-knot nematodes (*Meloidogyne* spp.) are considered among the most deteriorating soilborn parasites that can significantly affect many field crops, trees and turfgrass (Gill and Mcsorley, 2011). Nematodes are characterized with a broad host range of greater than 3,000 plant species (Reddy, 1985). Furthermore, it was reported that around 5% of the world crop production was lost annually due to infection with *Meloidogyne* species (Karajeh, 2008), the losses can reach up to 64% of the yield (Roberts et al., 2005; Sikora et al., 2007; Balbaa, 2010).

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 (Crow, 2005).

Meloidogyne species encompass 98 species, among them *M. incognita, M. javanica, M. hapla*, and *M. arenaria* are considered the most common (Jones et al., 2013).

After banning Nemacur in 2008 due to environmental concerns, there is a dire need for developing new efficient alternatives to control such plant-parasitic nematodes. In this regard, the narrow range effectiveness characterizing biological control agents limits it`s its applicability. For example, the bacterial parasite, *Pasteuria sp* sp. can control sting nematodes (*Belonolaimus longicaudatus*) (Luc et al., 2010); however, it does can not affect the other species of plant-parasitic nematodes such as root knot nematodes (*Meloidogyne* spp.).

By virtue of the well-established nematicidal effect of silver nanoparticles (AgNPs) (Roh et al., 2009; Lim et al., 2012), AgNPs was proposed (Cromwell et al., 2014)) as a potential alternative nematicide.

In this regard, many papers have established a robust emphasis on the antimicrobial effect of copper nanoparticles (CuNPs) (Karthik and Geetha, 2013; Betancourt-Galindo, 2014; Viet et al., 2016); thus, in this paper, we evaluate the *In vitro* nematicidal efficiency of CuNPs against J2 of *M. incognita* as another potential alternative for controlling such parasite.

MATERIALS & METHODS

Preparation of copper nanoparticles:

Copper nanoparticles (CuNPs) were prepared according to the chemical reduction method (Biçer et al., 2010). In this method, L-ascorbic acid (Future Modern Co., Egypt.) was used as a reducing agent, in the presence of Cetyl trimethylammonium bromide (CTAB) (Sigma-Aldrich, Egypt.) as a cationic surfactant, to reduce copper cations provided from copper sulfate pentahydrate (Elnasr Pharmaceuticals Co., Egypt) 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. Copper nanoparticles were centrifugally collected for further characterization and application.

Characterization of Copper Nanoparticles:

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 copper nanoparticles:**

300 cm^3 jars 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. All jars were incubated at room temperature for 3 days. After the said mentioned exposure time, nematodes were extracted, counted and mortality was calculated.

Statistical analysis

SPSS 22 software 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 (McDonald, 2008).

RESULTS

Confirming the Synthesis of copper nanoparticles:

Successful synthesis of copper nanoparticles was confirmed through exhibiting their characteristic surface plasmon resonance peak which was detected using Uv-Vis Spectrophotometer (ORION AQUAMATE 8000) at wave length of 572 nm, as shown in Figure (1).

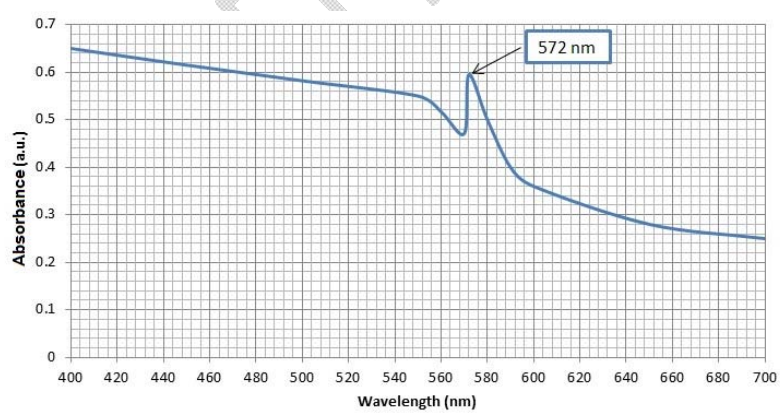

Figure (1): characteristic surface plasmon resonance peak of copper nanoparticles at 572 nm.

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

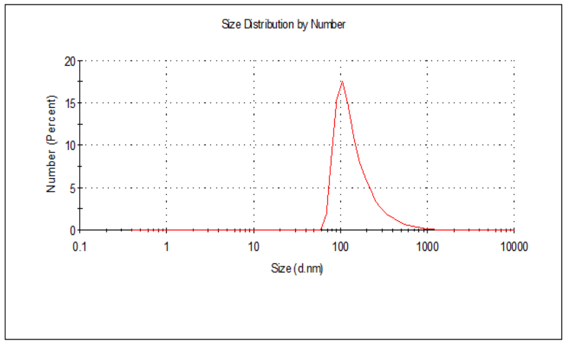

Figure (2): Particle size distribution by number of CuNPs, showing the average particle size of about 100 nm.

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

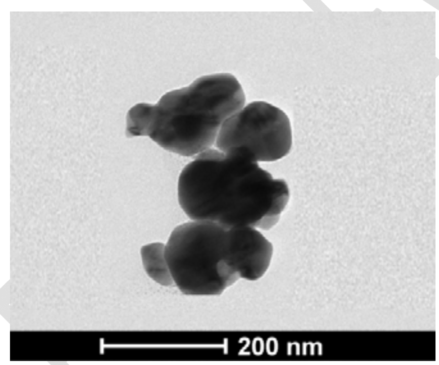

Figure (3): Transmission Electron Micrograph of the synthesized CuNPs showing the spherical shape of the particles.

Evaluation of the nematicidal effect of copper nanoparticles:

Statistical analysis showed that all concentrations of CuNPs exhibited significant inhibitions on the J2 *M. incogneta*. In this regard, 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-dependency mortality of *M. incognita* caused by CuNPs can be shown in Figure (5).

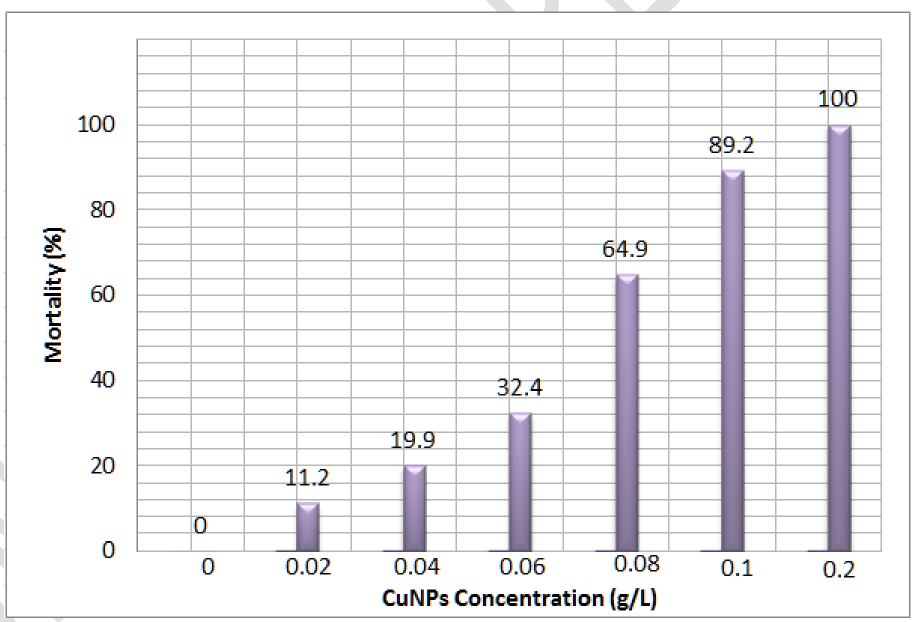

Figure (5): a graph shows the linear nematicidal effect of copper nanoparticles.

DISCUSSION

This study has emphasized on the potential *In vitro* nematicidal effect exhibited by copper nanoparticles 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 copper nanoparticles compared with non-treated control.

Among different types of nanoparticles, the nematicidal effect of silver nanoparticles has extensively studies; but, from this investigation, it is noteworthy that copper nanoparticles could exhibit a significantly higher nematicidal effect than silver nanoparticles 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 (Entsar Taha, 2016). On the other hand, CuNPs at the same concentration could achieve a mortality of 100% after 3 days of indirect exposure in soil. This may due to the profound toxicological effect of copper nanoparticles in DNA damage, this in contrast to the more mild effect of AgNPs, which depended 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 (Lok et al., 2006; Choi and Hu, 2008) and eukaryotes (Ahamed et al., 2010; Lim et al., 2012).

In addition, it was reported (Hassan et al., 2016) that the highest percentage of mortality achieved after 3 days of direct exposure of second stage juveniles (J2) to silver nanoparticles was 95%; while higher mortality percentage (100%) was attained using copper nanoparticles, despite the indirect exposure.

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.

To sum up all, it can be said concluded that copper nanoparticles may provide an alternative nematicidal effect against the root-knot nematodes, *M. incogneta* in many economic crops, trees and turfgrasses. But, further research should be conducted in order to investigate its environmental consequences, hence determining the optimum concentrations and doses that can be applied in field without considerable hazards.

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