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Effectiveness of Silver Nanoparticles against Root-Knot Nematode, *Meloidogyne incognita* Infecting Tomato under Greenhouse Conditions

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Abstract

Root-knot nematode, *Meloidogyne incognita* is the most economically important plant parasitic nematode species that cause serious damage to most agricultural crops including tomato worldwide. Nematicides do not provide long-term suppression of root-knot nematodes, and environmental and human health concerns are resulting in increased restrictions on their use. A greenhouse experiment was conducted to evaluate the effect of silver nanoparticles (AgNPs) at concentrations of 0.25, 0.5 and 1 mM as a potential nematicide on *M. incognita* infecting tomato. AgNP was biologically and chemically synthesized by a reaction of silver nitrate with ginger (*Zingiber officinale*) rhizomes aqueous extract and sodium borohydride, respectively. Results indicated that application of AgNPs improved plant growth and reduced nematode infection in comparison to silver nitrate and control treatments. The highest increment of fresh weight as well as the lowest numbers of galls and egg-masses was obtained when tomato plants was treated with AgNP produced by ginger extract at 1 mM.

Keywords: silver nanoparticles, *Meloidogyne incognita*, tomato, nematicide

1. Introduction

Plant parasitic nematodes caused significant damage to most agricultural crops reducing the yield and quality, causing loses valued at over $75 billion per annum in the tropical and sub-tropics (Luc et al., 2005). Worldwide, crop loss attributed to these pests could be estimated by 20.6% (Sasser & Freckman, 1987). Root-knot nematodes (RKNs, *Meloidogyne* species) have broad host plant specificity and are responsible for > US$125 billion annually in world-wide crop losses (Chitwood, 2003). *M. arenaria*, *M. hapla*, *M. javanica* and *M. incognita* are considered to be the most popular species that caused more than 90% of the estimated damages, they affected major both field and vegetable crops. The most damaging of all root-knot nematodes is the southern RKN, *M. incognita*, which infects almost all agricultural plants including tomato. Due to environmental restrictions on nematicidal use for controlling plant parasitic nematodes, biological control measures have gained increasing interest; however, there is still a need for alternative compounds for effective nematode control to be developed (Noling & Becker, 1994).

The development of nanotechnologies is now being observed worldwide. Nanotechnology has a great impact on biological sciences and more and more nanomaterials are used in medicine, pharmacy and agriculture (Myczko, 2006). Silver nanoparticles (AgNPs) have emerged as an arch product from the field of nanotechnology. Over the last few years due to its good conductivity, chemical stability, catalytic and antibacterial activity silver has gained much of the interest. Production of silver nanoparticles can be achieved through different methods (Hardman, 2006). Chemical approaches are the most popular methods for the production. However, some chemical methods cannot avoid the use of toxic chemicals in the synthesis protocol (Ahmad et al., 2003). To overcome this problem, researchers are moving towards the use of clean, nontoxic, harmless and environmentally friendly biological methods or “green” chemistry such as use of plant extracts. There are several reports that claimed that plant extracts have been recorded to posses nematicidal and nematostatic properties (Nour El-Deen & Darwish, 2011; Nour El-Deen et al., 2014; Khan et al., 2017; Singh et al., 2017).

Biosynthetic of metal (Ag, Au, Cu and Cd) nano-formulation of plant extracts has received an increasing attention because of their potential application in pest control.

AgNP has shown evidence of being a potentially effective nematicide (Roh et al., 2009), and its toxicity is associated with induction of oxidative stress in the cells of targeted nematodes (Lim et al., 2012). Ag-nano particles of *Urtica urens* extracts concomitant with rugby were effective in the management of *M. incognita*, since it increased nematicidal activity 11-fold more than the least toxic extract against eggs (Nassar, 2016). The toxicity of three nanoparticles, silver, silicon oxide and titanium oxide, to the root-knot nematode, *Meloidogyne incognita*, was recorded in laboratory and pot experiments (Ardakani, 2013). Although most researchers have investigated the antifungal, antiviral and antibacterial activities of AgNPs, little attention has been given to nematicidal activities of such material. Therefore, the aim of this study was to evaluate the effectiveness of biological and chemical Ag-nano formulations against the root-knot nematode, *M. incognita* under greenhouse conditions.

2. Materials and Methods

2.1 Plant Extract Preparation

Ginger (*Zingiber officinale*) rhizomes were purchased from local market, Taif, KSA. Rhizomes were washed with distilled water to remove debris and soil. Plant rhizomes were cut into small pieces, and dried in a vacuum oven for 3 h. A portion of 25 g was crushed in an electric blender with adding 200 ml of Milli-Q water during crushing. The extract was stirred and incubated at room temperature for 6 h, and filtered using a Whatman No 1 filter paper. The extract was stored at 4 °C until further use.

2.2 Silver Nanoparticles Preparation

For biological synthesis of silver nanoparticles, 90 ml of 1 mM $AgNO_3$ (in Milli-Q water) was taken in a sterile reaction bottle and 10 ml of aqueous plant extract was added to it. The solution was mixed well and kept in a shaker incubator for overnight at 37 °C. As a result, dark brown color solution was formed, indicating the formation of silver nanoparticles. AgNP was chemically synthesized according to (Fan et al., 2009) by adding 15 ml of 0.1 M $AgNO_3$ to 970 ml of 0.2% starch solution, and then 30 ml of 0.1 M NaBH4 was added into the reaction solution. After stirring for 30 min, a brown AgNP solution was appeared.

2.3 Characterization of Silver Nanoparticles

2.3.1 Visual Observations and UV-Vis Spectra Analysis

The biogenic and chemical synthesized of silver nanoparticles using plant rhizomes or sodium borohydride were characterized by UV-Vis spectroscopy (Perkin Elmer, Lambda 25) instrument scanning in the range of 200-900 nm, at a resolution of 1 nm (Klaus-Jeorger et al., 2001; Ahmad et al., 2003). All Samples were prepared by centrifuging an aliquot of plant extract or chemical filtrate (1.5 ml) at 10000 rpm for 10 min and diluted 10-fold for all experiments involving measurement of UV-Vis spectra. $AgNO_3$ solution without addition of plant extract or sodium borohydride was used as a control throughout the experiment.

2.3.2 Transmission Electron Microscopy (TEM)

Samples of silver nanoparticles for transmission electron microscopy (TEM) analysis were prepared on carbon-coated copper TEM grids. Studies of size, morphology and composition of the nanoparticles were performed by means of transmission electron microscopy (TEM) operated at 120 kV accelerating voltage (JTEM-1230, Japan, JEOL). Finally, the obtained images were processed using the software ImageJ. ImageJ developed at the National Institutes of Health (NIH), USA is a Java-based public domain image processing and analysis program (Rasband, 1997-2015).

2.4 Bioassay

Two-weeks-old tomato seedlings c.v. Super Strain B were transplanted into 15-cm-d. plastic pots (one seedling/pot) filled with 2000 g of nematode-infested sandy loam soil (approximatly 2 J_2/g of soil) collected from a pure culture of *M. incognita* that previously identified according to the characteristics of its perineal pattern (Taylor & Sasser, 1978), maintained and propagated on eggplant. Seedlings of tomato were allowed to grow for another 15 days, then twenty four pots were treated with 15 ml of AgNPs solutions at 0.25, 0.50 and 1.0 mM on the soil surface. Twelve pots were recieved silver nitrate ($AgNO_3$) as a positive control, whereas, four untreated seedlings were left to serve as a negative control. All plastic pots were randomly arranged on a greenhouse bench at 25±2 °C and watered regularly as needed. The seedlings were uprooted 45 days later. Data dealing with length of shoot and root, and fresh weights of shoot and root were measured. The total number of galls and egg-masses per root system were recorded and root galling (RGI) and egg-mass (EI) indices were recorded based on a scale of

1-9 where l = no gall or egg-mass, 2 = 1-5, 3 = 6-10, 4 = 11-20, 5 = 21-30, 6 = 31-50, 7 = 51-70, 8 = 71-100 and 9 ≥ 100 galls or egg-masses/plant (Sharma et al., 1994). The experiment had four replicates and was repeated once.

2.5 Statistical Analysis

Statistically, the obtained data were subjected to analysis of variance (ANOVA) (K. A. Gomez & A. A. Gomez, 1984) followed by Duncan's multiple range to compare means (Duncan, 1955).

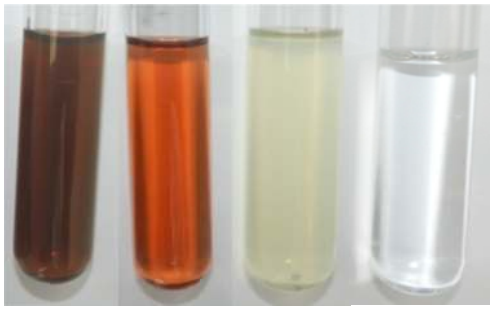
3. Results

3.1 Characterization of Silver Nanoparticles

In this work, biofabricated of AgNPs by plant rhizomes extract as well as chemical one was described. Visual observation of the reaction of plant extract or sodium borohydride with silver nitrate at room temperature showed a color change from colorless to yellowish or dark brown whereas no color change could be demonstrated in $AgNO_3$ alone (Figure 1). UV-Vis spectrum of the biosynthesis or chemical AgNPs is shown in the Figures 2 and 3, respectively. For biosynthesized AgNPs, Surface Plasmon peak observed at 450 nm, whereas, a peak at 430 nm corresponds to the characteristic wavelength of AgNPs synthesized by chemical method.

3.2 TEM Analysis

Figure 4 showed the representative TEM image, with a size distribution on its right side. The TEM images of AgNPs and their size distribution produced either by biological or chemical method showed that the particles were spherical, and monodispersed with average diameter of 5-50 nm.



1 2 3 4

Figure 1. Digital photograph of synthesized silver nanoparticles by incubated of plant rhizome extract or sodium borohydride with $AgNO_3$ solution for 24 h at 30 °C

Note. (1) biosynthesized AgNPs, (2) chemical AgNPs, (3) plant extract alone, and (4) $AgNO_3$ alone.

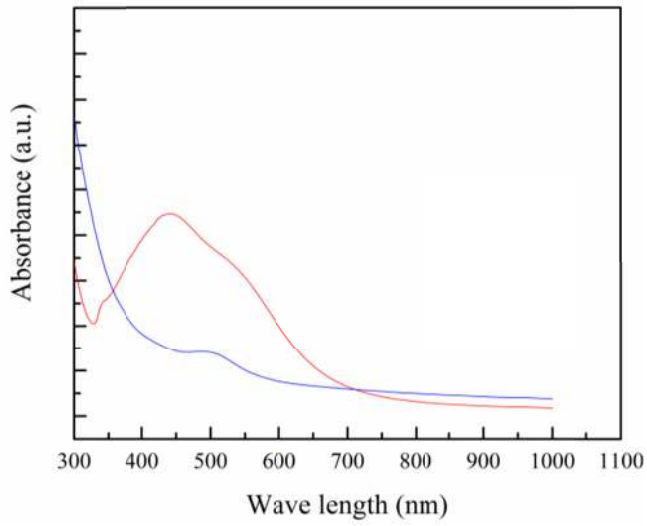


Figure 2. UV-Vis absorption spectrum of silver nanoparticles (AgNPs) synthesized using ginger extract

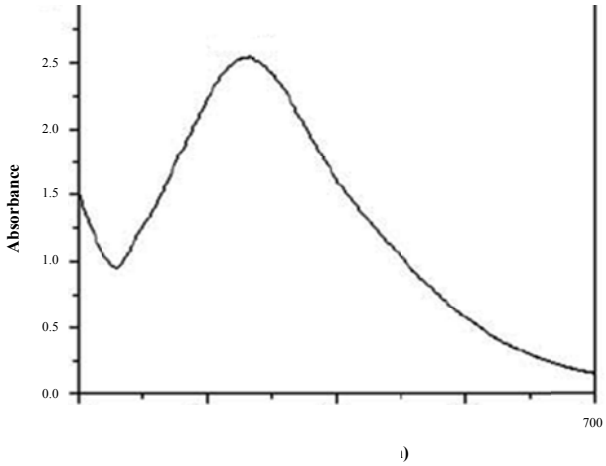


Figure 3. UV-Vis absorption spectrum of silver nanoparticles (AgNPs) synthesized using sodium borohydride

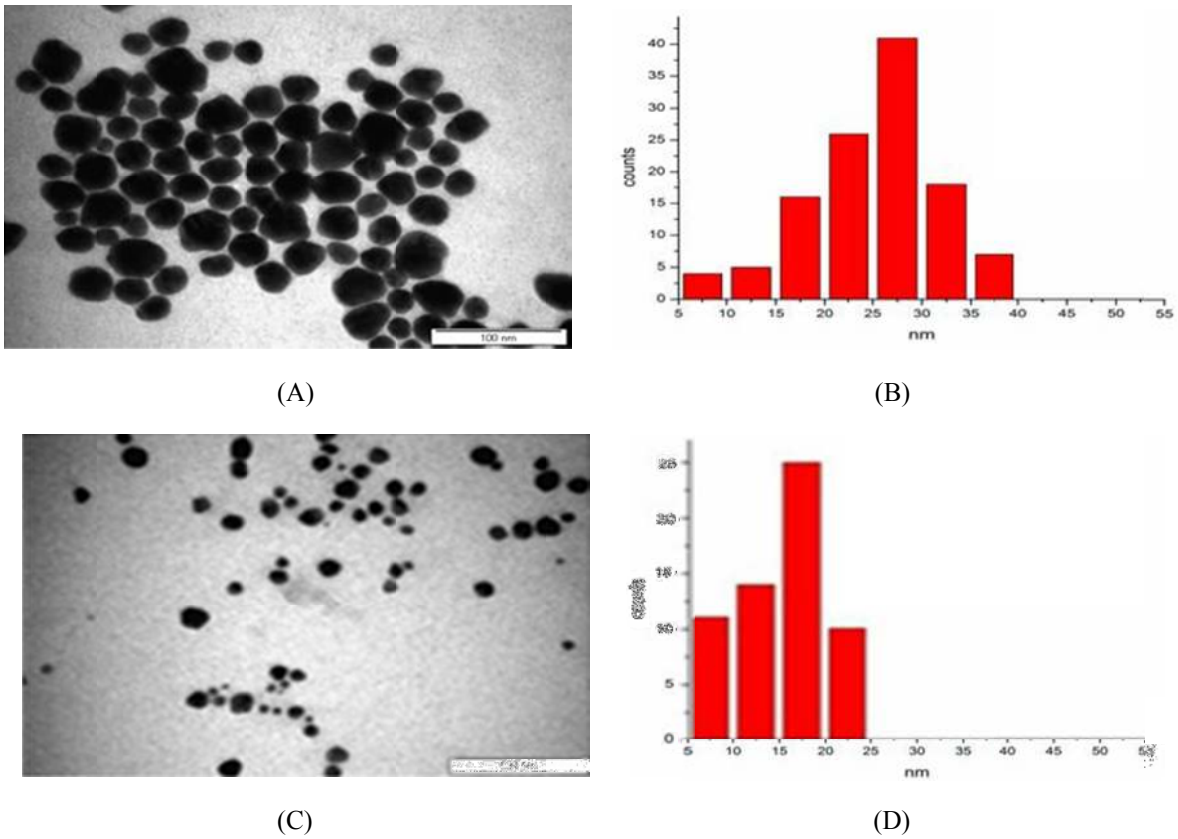


Figure 4. TEM images and particle size distributions of silver nanoparticles (AgNPs)

Note. AgNPs synthesized using plant extract (A & B) and sodium borohydride (C & D).

3.3 Bioassay

Data in the Table 1 represent the impact of biological and chemical synthesized AgNPs on the growth of tomato infected with *M. incognita* in comparison to $AgNO_3$. Results indicated that all treatments caused remarkable improvement in tomato growth in terms of shoot and root length and fresh weight of shoot and root with various degrees as compared to control. Among all tested materials, chemical AgNP at 0.50 mM significantly gave the highest increment in shoot and root lengths with values of 93 and 18 cm, respectively. However, nanoparticles of Ag-ginger at high concentration did not significantly influence ($P \leq 0.05$) the growth of tomato cv. Super Strain B, determined by root length (Table 1). Fresh weights of tomato plants grown in untreated soil were significantly

less than those grown in AgNP-treated soil. The weight of tomato plants grown in untreated soil was 16.3 g, whereas those from soils treated with nanoparticles of Ag-ginger at 1.0, 0.50 and 0.25 mM were 55.4, 52 and 48.2 g, respectively. Likewise, moderate increment was obtained by the application of $AgNO_3$ at 0.25 and 0.50 mM (37.5 and 33.6 g), whereas, among all treatments, $AgNO_3$ at higher concentration gave the least value of fresh weight that averaged 20.9 g.

Data in the Table 2 reveal number of root galls, root gall index (RGI), number of egg-masses and egg-mass index (EI) of *M. incognita* infecting tomato as influenced by AgNPs those either chemically or biologically prepared. Significant results were noticed between all tested materials and nematode alone (control) with the indices of root galls and egg-masses number, since their values were ranged between 1.3 to 6 vs 7 for root galls and 1 to 4 vs 5 for egg-masses, respectively. The incidence of *M. incognita* infection of tomato plants was not affected by any of tested compounds, but the root galling was significantly reduced ($P \leq 0.05$) by all treatments when compared with the non-treated one (Table 2).

Roots of tomato plants grown in Ag-ginger nanoparticles treated soil had very few or no galls, while control plants had heavily galled roots (Figure 5). The number of nematode egg-masses per plant was also significantly reduced by AgNPs treatments: in particular, no egg-masses were formed on tomato roots grown in soil amended with biological AgNPs at all concentrations tested.

Table 1. Effect of chemical and biological AgNPs on the growth of tomato seedlings infected with *M. incognita* under greenhouse conditions

Treatments	Conc. (mM)	*Plant growth response				
		Length (cm)		Fresh weight (g)		Fresh wt. of the whole plant (g)
		Shoot	Root	Shoot	Root	
Biological AgNP	0.25	86.3 b	16 ab	45.3 b	2.9 cd	48.2 c
	0.50	75.7 cd	13 c	46.7 b	5.3 a	52 b
	1.0	52 f	13 c	50.8 a	4.6 a	55.4 a
Chemical AgNP	0.25	79.3 c	16 ab	37.3 c	3.8 b	41.1 d
	0.50	93 a	18 a	34.6 c	3.3 bc	37.9 de
	1.0	75 d	16 ab	21.3 e	2.1 ef	23.4 g
$AgNO_3$	0.25	63 e	14 bc	35.3 c	2.2 ef	37.5 e
	0.50	65 e	16 ab	31 d	2.6 de	33.6 f
	1.0	50 f	16 ab	19.3 e	1.6 fg	20.9 g
Control (Check)		35 g	12 c	15 f	1.3 g	16.3 h
LSD at 0.05		4.2	2.6	2.9	0.7	3.2

Note. Each value represents the mean of four replicates. Within each concentration, values with the same letter do not differ significantly according to LSD ($P \leq 0.05$).

Table 2. Effect of soil treatment with AgNPs on galls and egg-masses of tomato (index of 0 to 9) caused by *M. incognita* under greenhouse conditions

Treatments	Conc. (mM)	No. of Galls	RGI	No. of Egg-masses	EI
Biological AgNP	0.25	4.7 cd	2 ef	0 f	1 d
	0.50	2 d	2 ef	0 f	1 d
	1.0	1.3 d	1.3 f	0 f	1 d
Chemical AgNP	0.25	6 cd	2.7 de	1 ef	1.7 cd
	0.50	10.3 c	3.3 d	3.7 de	2 c
	1.0	20.7 b	4.3 c	5.3 d	2.3 c
$AgNO_3$	0.25	25 b	5 c	12 c	4 b
	0.50	28 b	5 c	14.7 bc	4 b
	1.0	48 a	6 b	16 b	4 b
Control (Check)		55 a	7 a	24 a	5 a
LSD at 0.05		8.01	0.85	2.9	0.72

Note. Each value represents the mean of four replicates. Within each concentration, values with the same letter do not differ significantly according to LSD ($P \leq 0.05$).

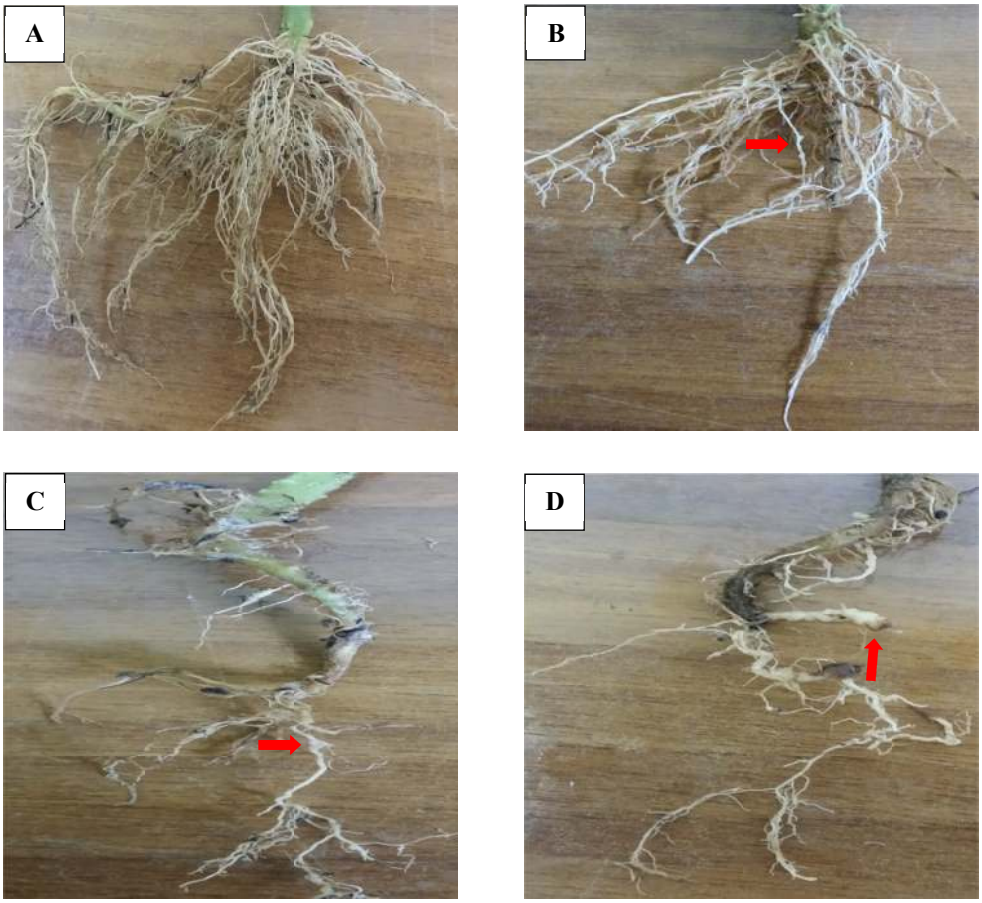


Figure 5. Roots of tomato plants grown in nematode-infested soil treated or untreated

Note. (A) Ag-ginger NP, (B) Chemical AgNP, (C) $AgNO_3$ at a concentration of 1.0 mM, and (D) untreated soil (control). Arrow indicates to root galls.

4. Discussion

Apparently, the presence of spherical, monodispersed and small particles in TEM image is in accordance with the UV-Vis spectral study (Ahmad et al., 2003). It is well known that silver nanoparticles exhibit yellowish brown color in aqueous solution due to excitation of Surface Plasmon vibrations in silver nanoparticles (Jain et al., 2009).

In agreement with previous reports, the absorption peak at 455 nm is probably due to the excitation of longitudinal plasmon vibrations and formation of quasi-linear superstructures of nanoparticles (Mukherjee et al., 2001). Herein, application of nanoparticles those chemically or biologically prepared against *M. incognita* infecting tomato seedlings c.v. Super Strain B under greenhouse conditions significantly suppressed nematode population in return enhancement the plant growth parameters.

Data also revealed that Ag-ginger-NP significantly showed the highest increment in plant growth parameters in terms of shoot and root weights followed by chemical AgNP then $AgNO_3$ for the same previous growth characters. Moreover, the greater and significant increase percentages in shoot and root lengths were recorded by the application of chemical AgNPs. The present findings agreed with that of Nassar (2016) in respect to nematicidal activity of Ag-nano particles of *Urtica urens* extracts concomitant with rugby against *M. incognita* in-vitro and silver, silicon oxide and titanium oxide nanoparticles to the root-knot nematode, *M. incognita* in laboratory and pot experiments (Ardakani, 2013). It is worth to note that although Ag-nano of ginger extract showed the highest reduction of nematode gall formation and egg-mass production, conversely it gave shorter roots in comparing to other treatments. A positive correlation was observed between root galling and chemical AgNP concentrations, since increasing concentration increased RGI, whereas, negative correlation was noticed regarding biological AgNP concentration. Our findings are in line with several reports that claimed that toxicity

of sublethal doses of AgNP to nematodes can result in reproduction inhibition [with 0.05 to 0.5 mg/ml of AgNP for 72 h (Roh et al., 2009; Lim et al., 2012)] or growth inhibition [with 5 to 50 mg/ml of AgNP for 1 to 3 d (Meyer et al., 2010)]. This suggests the AgNP effect may be subtle and chronic at low concentrations applied in the field. Nematicidal effect of AgNP against root-knot nematodes likely applies to other genera of plant-parasitic nematodes and also to plant-pathogenic fungi, because its mode of action is not specific but associated with disrupting multiple cellular mechanisms including membrane permeability, ATP synthesis, and response to oxidative stress in both eukaryotic (Roh et al., 2009; Ahamed et al., 2010; Lim et al., 2012) and prokaryotic cells (Sondi & Salopek-Sondi, 2004; Morones et al., 2005; Lok et al., 2006; Choi & Hu, 2008). Results obtained from the present study clearly suggested that Ag-nano of ginger extract showed great potential in the inhibition of *M. incognita* development and improvement of tomato growth. On the other hand, $AgNO_3$ application did not act as strong nematicide on nematodes, since it was found to be the least effective to enhance plant growth parameters as well as nematode development. These findings could be important from the point of view of using novel ecofriendly method to control the root-knot nematode without the use of chemical pesticides under greenhouse condition.

5. Conclusion

Conclusively, utilization of such technique in root-knot nematode control could gain new trend, safe and effective nematode management program. Therefore, further studies are needed to prepare and characterize biofabricated nanoparticle that are nematotoxic and possessing complex modes of action before recommend it for field application and IPM program against plant parasitic nematodes.

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