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Nematicidal Effects of Silver Nanoparticles on Root-knot Nematodes (*Meloidogyne incognita*) in laboratory and screenhouse

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ABSTRACT

In this investigation, silver nanoparticles (AgNP) were evaluated as a nematicidal substance in laboratory and screenhouse experiments. Second infective juveniles (IJ2) of *Meloidogyne incognita* were exposed to AgNP in water at with the consternations of 20, 40, 200, 500, and 1500 ppm/ml. the concentration of 200 ppm caused 52% mortality at the third day, while 500 ppm caused 51% mortality after one day and 64%, 82% after the second and the final day, respectively. The most effective concentration was 1500 ppm which caused 89%, 93, and 96.5 %, respectively. In the screen house experiment, all the concentrations of AgNP inhibited the nematode growth (gall and egg formation and final population) and eggs hatchability. However the high concentrations of 200 ppm, 500 ppm, and 1500 ppm were more significant in their effect. The application of AgNP didn't show toxic effect on the plant growth or the free living nematodes. It can be concluded that, the laboratory assays to the nematicidal effect of AgNP, and the screenhouse evaluation demonstrated that, its nano silver may be a more safety alternative method to control root knot nematodes.

Keywords: *Meloidogyne*, *incognita*, silver nanoparticles, Tomato, management.

INTRODUCTION

Plant-parasitic nematodes are serious pests which cause significant damage to several host plants (Crow, 2007). Vegetables damage caused by nematodes can be economic. The recent major issue concerning nematode damage to host plants is the lack of effective chemical treatment methods for nematodes, effective alternatives are limited. Bio-control agents have disadvantages, such as the narrow range of treatment effects and lack of accuracy in the field application because of varying environmental conditions. For example, *Pasteuria* sp. that is a bacterial parasite of the sting nematode (*Belonolaimus longicaudatus*) (Luc *et al*., 2010) active in the laboratory trails while the commercial formulation of it was ineffective for the plant-parasitic nematodes such as sting nematodes or root- knot nematodes (Crow et al., 2011).

Silver nanoparticles (AgNP) are nano- materials being applied as an active ingredients of controlling human pathogenic microbes, such as bacteria and fungi (Kim *et al*., 2007; Furno *et al*., 2004; Yin *et al.,* 1999 and Wright *et al*., 1999) and used as a nematicide (Roh *et al.,* 2009), and its toxicity due to induction of oxidative stress in the cells of targeted nematodes (Lim *et al*., 2012).

The aim of this study was to investigate the potential of AgNP as a nematicide for *Meloidogyne incognita* in the laboratory assays and on tomato plants in the screen house.

MATERIALS AND METHODS

A- Materials

1- Root-knot nematode culture:

Culture of *Meloidogyne incognita* (Mi) was maintained in the screenhouse on tomato plants (*Lycopersicon esculentum* L. var. Castel Rock) in sterile sandy loam soil. IJ2 were extracted from tomato galled roots; washing, cutting into pieces and placed in the mist chamber for egg hatching. Hatched J_2s were collected daily and refrigerated at 6 ^{o}C for the experimental use.

2- The host plant:

Screenhouse experiments were carried out using 21-day-old tomato (*Lycopersicon esculentum* L. var. Castel Rock) seedlings. They were transplanted singly in 25-cm-diam pots which filled with sterilized sandy clay soil.

3- Silver nanoparticle preparation:

AgNP used in this study was chemically produced (Fan *et al.*, 2009) at the levels of 20, 40, 200, 500, and 1500 ppm were prepared to use.

4- Statistical analysis

The data of all experiments were statistically analyzed using analysis of variance procedure proposped by Snedecor and Cochran (1969). The differences between means were compared using Duncan's Multiple Range Test (Duncan, 1955).

B- Experimental methods:

1-Effect of different concentrations of nano silver on the survival of the second stage larvae (J_2s) of *M. incognita*:

About 50 IJ2 nematodes were added to 1ml of solutions containing 0, 20, 40, 50, 500, or 1500 ppm/ml of AgNP, with five replicates of each concentration. They were incubated at incubator of 25±1˚C. At the end of the 1st, 2nd, and the third day, alive nematodes were counted using the compound microscope and corrected mortality percentages were calculated. Healthy nematodes were defined as those were curled whereas unhealthy nematodes were defined as those appeared stiff- or straight-bodied. Alive nematodes were washed to use in the screenhouse experiment.

1-Effect of different concentrations of nano silver on the pathogenicity and reproductivity of *M. incognita* on tomato seedlings and on the free living nematodes:

The following experiment was conducted in 25 cm. pots under outdoor screen-house conditions. All treatments of all experiments were replicated five times. Tomato seedlings were transplanted in pots containing sterilized sandy loam soil. 25 pots were saturated with each one of the previous concentrations of AgNP. the 25 pots were divided into five groups as follow: the First, didn't receive nematodes, the second, received 1000 J_2s of untreated Mi at the same time with AgNP, while the third, received 1000 J_2s of untreated Mi after one week of AgNP application. the fourth, received 1000 J_2s of the treated Mi at the same time with AgNP, The last one, received 1000 J_2s of the treated Mi after one week of AgNP application. Check treatments were represented by untreated AgNP-pots supplied with nematodes only, and neither AgNP nor nematode. The experiment was ended after 60 days.

RESULTS

1-Exposure of Mi to silver nanoparticles in water:

AgNP decreased the activity of Mi. as concentration of AgNP increased and the data was correlated to the exposure time only the highest concentration (200 ppm, 500 ppm, and 1500 ppm) caused more than 50 % mortality, 200 ppm caused 52% mortality in the last day, while 500 ppm caused 51% mortality after the first day and 64%, 82% after the second and the final day respectively. The most effective concentration was 1500 ppm which caused 89%, 93%, and 96.5 %, respectively after the three days. (Fig. 1).

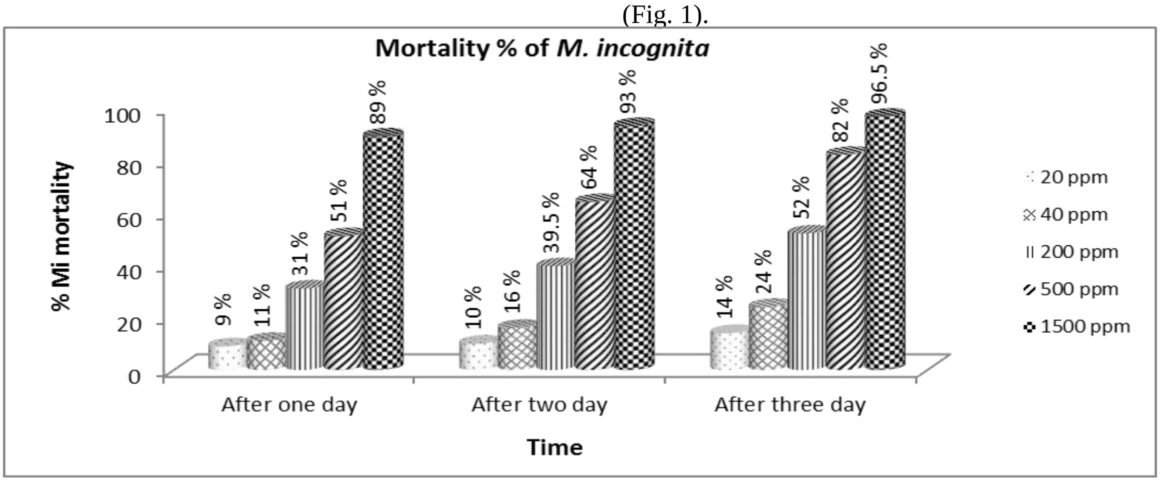

Fig. (1): The effect of different concentrations of AgNP on *M. incognita* corrected mortality percentages.

2-Soil treatment with silver nanoparticles:

Numbers of *M. incognita* J2 extracted from soil samples were reduced when the soil was treated with AgNP, and there was an interaction between AgNP concentration and these reduction. Lower concentrations (20, and 40 ppm/ml) of AgNP reduced the number of J2 extracted from the soil, galls and eggs formation but not significantly while the rate of reproduction reduced significantly compared to the nontreated plots (Table 1, 2).

The concentrations 200.500 ppm were more effective in all nematode parameters especially gall formation and the final population (Table 3,4).

While the most effective concentration which effected significantly on all the nematode parameters was 1500 ppm of AgNP (Table 5).

Table (1): Effect of 20 ppm concentration of nano silver with or without *Meloidogyne incognita* on tomato plant parameters and nematode parameters.

20 ppm	Fresh shoot (gm)	Fresh root (gm)	length (cm)	No. of leaves	No. of flowers	Eggs	Galls	Pf	Rr	free living
Without Mi	10.8^{ab}	2.5^{d}	41.5^{a}	11.5^{ab}	9.0^{ab}	-	-	-	-	1756^{b}
Fresh same time	8.3^{b}	3.5^{c}	36.5^{abc}	12.8^{a}	7.0^{b}	20000^{bc}	169^{b}	2230^{b}	1.7^{bc}	1613^{b}
Fresh after week	11.3^{a}	5.5^{a}	34.5^{bcd}	12.5^{a}	9.3^{ab}	11657^{d}	106^{c}	1727^{bc}	1.1^{c}	1880^{b}
Treated same time	8.0^{b}	3.8^{bc}	33.2^{cd}	8^{b}	7.3^{ab}	25250^{b}	172^{b}	1320^{c}	2.2^{b}	2860^{a}
Treated after week	10.5^{ab}	2.3^{d}	34.5^{bcd}	9^{b}	10.8^{a}	13620^{cd}	155^{b}	1068^{c}	1.3^{c}	3680^{a}
Control (-)	9.4^{ab}	6.1^{a}	28.9^{d}	9.1^{b}	4.9^{c}	-	-	-	-	3433^{a}
Control (+)	12.3^{a}	4.5^{b}	40.5^{ab}	12.3^{a}	6.3^{bc}	50673^{a}	216^{a}	3460^{a}	3.5^{a}	3733^{a}

***Data followed by the common letters within a column are not significantly different.**

Table (2): Effect of 40 ppm concentration of nano silver with or without *Meloidogyne incognita* on tomato plant parameters and nematode parameters.

40 ppm	Fresh shoot (gm)	Fresh root (gm)	length (cm)	No. of leaves	No. of flowers	Eggs	Galls	Mi Pf	Rr of Mi	free living
Without Mi	13.5^{a}	5.5^{ab}	45.0^{ab}	12.3^{a}	10.8^{b}	-	-	-	-	3987^{ab}
Fresh same time	9.3^{b}	4.3^{abc}	33.8^{cd}	7.3^{cd}	0.8^{e}	10173^{b}	124^{c}	1703^{b}	1.7^{b}	4127^{ab}
Fresh after week	12.5^{a}	5.3^{ab}	39.0^{bc}	9.3^{bc}	12.5^{ab}	9457^{b}	92^{d}	1077^{b}	1.1^{b}	5033^{a}
Treated same time	6.0^{c}	5.3^{ab}	26.8^{d}	6.8^{d}	0.3^{e}	12750^{b}	154^{b}	1707^{b}	1.7^{b}	2850^{c}
Treated after week	13.0^{a}	3.8^{c}	51.3^{a}	11.0^{ab}	13.8^{a}	10110^{b}	108^{cd}	1243^{b}	1.2^{b}	2963^{c}
Control (-)	9.4^{b}	6.1^{a}	26.9^{d}	9.1^{bc}	4.9^{d}	-	-	-	-	3433^{abc}
Control (+)	12.3^{a}	4.5^{abc}	40.5^{bc}	12.3^{a}	6.3^{c}	50673^{a}	216^{a}	3460^{a}	3.5^{a}	3733^{abc}

***Data followed by the common letters within a column are not significantly different.**

Table (3): Effect of 200 ppm concentration of nano silver with or without *Meloidogyne incognita* on tomato plant parameters and nematode parameters.

200 ppm	Fresh shoot (gm)	Fresh root (gm)	length (cm)	No. of leaves	No. of flowers	Eggs	Galls	Mi Pf	Rr of Mi	free living
Without Mi	9.8[a]	2.8[c]	42.8[a]	8.8[bc]	4.0[ab]	-	-	-	-	3667[ab]
Fresh same time	6.5[b]	5.0[ab]	25.8[c]	10.3[b]	4.3[ab]	7367[bc]	114[b]	943[b]	0.9[b]	2200[c]
Fresh after week	9.5[a]	4.0[ab]	26.8[c]	10.8[ab]	2.8[b]	3666[a]	74[c]	697[b]	0.7[b]	3987[ab]
Treated same time	6.3[b]	4.5[ab]	31.8[bc]	7.5[c]	2.0[b]	9667[bc]	108[b]	1097[b]	1.1[b]	4797[a]
Treated after week	9.8[a]	2.5[c]	39.8[ab]	8.8[bc]	2.0[b]	8083[bc]	87[c]	727[b]	0.7[b]	3273[b]
Control (-)	9.4[a]	6.1[a]	26.9[c]	9.1[bc]	4.9[ab]	-	-	-	-	3433[ab]
Control (+)	12.3[a]	4.5[ab]	40.5[ab]	12.3[a]	6.3[a]	50673[a]	216[a]	3460[a]	3.5[a]	3733[ab]

***Data followed by the common letters within a column are not significantly different.**

Table (4): Effect of 500 ppm concentration of nano silver with or without *Meloidogyne incognita* on tomato plant parameters and nematode parameters.

500 ppm	Fresh shoot (gm)	Fresh root (gm)	length (cm)	No. of leaves	No. of flowers	Eggs	Galls	Mi Pf	Rr of Mi	free living
Without Mi	9.8[b]	3.8[b]	36.8[ab]	4.0[d]	7.8[a]	-	-	-	-	4797[a]
Fresh same time	7.8[bc]	4.3[ab]	28.8[c]	11.3[ab]	3.8[c]	1470[b]	48[b]	701[b]	0.7[b]	2200[c]
Fresh after week	8.5[bc]	4.5[ab]	34.3[bc]	8.3[c]	2.0[c]	855[b]	42[b]	410[b]	0.4[b]	4235[a]
Treated same time	7.3[c]	4.3[ab]	30.5[c]	10.5[ab]	5.8[ab]	2400[b]	53[b]	709[b]	0.7[b]	3436[b]
Treated after week	8.8[bc]	3.0[b]	37.3[ab]	8.3[c]	3.0[c]	1410[b]	58[b]	730[b]	0.7[b]	3520[b]
Control (-)	9.4[b]	6.1[a]	26.9[c]	9.1[bc]	4.9[bc]	-	-	-	-	3433[b]
Control (+)	12.3[a]	4.5[ab]	40.5[a]	12.3[a]	6.3[a]	50673[a]	216[a]	3460[a]	3.5[a]	3733[ab]

***Data followed by the common letters within a column are not significantly different.**

Table (5): Effect of 1500 ppm concentration of nano silver with or without *Meloidogyne incognita* on tomato plant parameters and nematode parameters.

1500 ppm	Fresh shoot (gm)	Fresh root (gm)	length (cm)	No. of leaves	No. of flowers	Eggs	Galls	Mi Pf	Rr of Mi	free living
Without Mi	10.8[ab]	3.5[c]	42.3[ab]	8.8[c]	3.5[bc]	-	-	-	-	10218[a]
Fresh same time	8.8[bc]	3.5[c]	34.0[c]	10.8[ab]	4.8[bc]	397[b]	36[b]	227[b]	0.2[b]	6385[abc]
Fresh after week	9.8[bc]	4.8[ab]	37.3[bc]	8.8[c]	3.0[bc]	256[b]	27[b]	146[b]	0.1[b]	8157[ab]
Treated same time	8.3[c]	4.3[bc]	37.5[abc]	11.3[a]	4.3[bc]	616[b]	39[b]	517[b]	0.5[b]	7527[abc]
Treated after week	12.8[a]	5.5[a]	42.5[a]	11.8[a]	8.0[a]	410[b]	36[b]	160[b]	0.2[b]	8488[ab]
Control (-)	9.4[bc]	6.1[a]	26.9[d]	9.1[bc]	4.9[bc]	-	-	-	-	3433[c]
Control (+)	12.3[a]	4.5[ab]	40.5[ab]	12.3[a]	6.3[ab]	50673[a]	216[a]	3460[a]	3.5[a]	3733[c]

***Data followed by the common letters within a column are not significantly different.**

As shown in tables 1,2,3,4,and 5 the free living nematodes were affected by the nano silver concentrations positively; the concentrations (20 ppm ,40 ppm, 200 ppm, 500 ppm) of AgNP increased the free living nematodes and the highest effect was obtained with the concentration (1500 ppm). Data in tables demonstrated that, adding the silver nano particles increased the plant growth (even when adding nematode infection with the AgNP) or at least was equal to which obtained in the control.

DISCUSSION

This study provided a proof that AgNP have the ability for management of root-knot nematodes and these data agreed with Cromwell *et al*. 2014. Inactivation of *M. incognita* J2 by direct exposure, and reduction of *M. incognita* J2 in soil treatments demonstrated the high nematicidal effects of AgNP, subsequently, the screenhouse trial demonstrated the beneficial effects of AgNP for intensively managed of nematodes without harming the free living nematodes or the plant growth, and so, determined to be safe for plants. In addition to that, nematicidal effect of AgNP against root-knot nematodes is not the same on other plant-parasitic nematodes and so to plant-pathogenic fungi, Moreover, only the high concentrations of nano silver are toxic to entomopathogenic nematodes, *Heterorhabditis indica* , *Steinernema arenarium* and *Steinernema abbasi* (Taha and Abo Shady, 2016), and *Caenorhabditis elegans* (Meyer *et al*., 2010; Lim *et al*., 2012). These effects may be due to that, its mode of action is not specific but associated with multiple cellular mechanisms including ATP synthesis, membrane permeability, and response to oxidative stress in both of eukaryotic cells (Roh *et al*., 2009; Ahamed *et al*., 2010; Lim *et al*., 2012) and prokaryotic cells (Sondi and Salopek-Sondi, 2004; Morones *et al*., 2005; Lok *et al*., 2006; Choi and Hu, 2008). For this reason, AgNP is a wide-spectrum antimicrobial agent which capable of affecting plant-pathogenic fungi and bacteria (Park *et al*., 2006; Jo *et al*., 2009). For example, it is possible that AgNP has an antifungal effect on several root-associated fungal pathogens (e.g., *Gaeumannomyces graminis* and *Rhizoctonia solani*) treated with AgNP may become more tolerant to root-knot nematode damage because of some protection from additional stress by these other pathogens. Therefore, AgNP may provide an additional benefit of managing multiple plant pathogens. The lethal effect was confirmed by the soil treatment with AgNP that effectively reduced IJ2 that could be extracted from the soil, galls and eggs formation However, even though plant quality was improved by the AgNP treatment.

Toxicity of sub lethal doses of AgNP to nematodes could result in reproduction inhibition with 20 ,40 ppm/ml of AgNP, (Meyer *et al*., 2010) obtained the same data up to 50 ppm. This suggests the AgNP effect may be subtle and chronic at low concentrations applied in the field and this is agreed with the present data. AgNP has a nematicidal activity which may provide an alternative to high-risk chemical nematicides.

In conclusion this research, emphasized positive efficacy of AgNP against root-knot nematodes (*Meloidogyne incognita*) in tomato without phytotoxicity on the plant growth or the non parasitic nematodes.

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التاثير الابادى لجزيئات النانو فضة على نيماتودا تعقد الجذور فى المعمل وعلى الطماطم فى الصوبة الزراعية

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تعتبر النيماتودا الممرضة للنبات أحد الافات الاقتصادية على محاصيل الخضر ومنها الطماطم، وتعد نيماتودا تعقد الجذور احد اهم اجناس النيماتودا التى تصيب محصول الطماطم. ونظرا للمخاطر الصحية والبيئية للمبيدات النيماتودية فقد اتجهت الانظار لمحاولة ايجاد بدائل قد تكون اكثر امان. تناول البحث دراسة تأثير تركيزات متصاعدة من جزيئات النانو فضة (٢٠, ٤٠, ٥٠٠, ١٥٠٠ ml /ppm) على حيوية نيماتودا تعقد الجذور فى اطباق بترى لمدة ثلاثة ايام فى المعمل و فى الصوبة على نباتات الطماطم لمدة ٦٠ يوم. واظهرت النتائج المعملية ما يلى: أحدث التركيز 200 ppm فى نسبة موت ٥٢% بعد اليوم الثالث، فى حين أحدث التركيز 500 ppm فى نسب موت ٥١%و ٦٤%و ٨٢% بعد اليوم الاول والثانى والثالث على الترتيب، فى حين كان تأثير التركيز 1500 ppm عالى المعنوية من اليوم الاول حيث سجل نسب موت ٨٩%و ٩٣%و ٩٦.٥% بالترتيب. على الجانب الاخر اظهرت نتائج الصوبة ما يلى: أحدثت جزيئات النانو فضة تأثير مثبط لنمو النيماتودا وتطورها على نباتات الخيار كما اظهرت تأثير مثبط لفقس البيض، ولكن كانت المعنوية مرتفعة فى التركيزات العالية من جزيئات النانو. كما انه كان تأثير الفضة منشط لنمو النباتات وخاصة فى التركيزات العالية حيث لم يتأثر نمو النباتات بالاصابة بالنيماتودا. كما أن جزيئات الفضة اظهرت تأثير منشط أو على الاقل غير مثبط على النيماتودا حرة المعيشة. وعليه فانه يمكن استخلاص ما يلى: يمكن الاعتماد على جزيئات النانو فضة كبديل للمبيدات النيماتودية حيث يعتبر أكثر أمان لصحة الانسان والبيئة وذلك دون الخوف على النباتات أو النيماتودا حرة المعيشة.