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Research Article
Effect of Silver Nanoparticles on the Mortality Pathogenicity and Reproductivity of Entomopathogenic Nematodes

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Abstract

Background and Objective: Scientists are concerning about using nanomaterials, such as silver nanoparticles (AgNP) in biological control of plant parasitic nematodes. In this study, it was evaluate that the side effect of AgNP on the non target nematodes, entomopathogenic nematodes (EPNs) that found naturally in the same soil environment and contribute to the insect pests control. **Methodology:** The effect of different concentrations (1500, 500, 200, 40 and 20 ppm) of silver nanoparticles on mortality of EPNs *Heterorhabditis indica*, *Steinernema arenarium* and *Steinernema abbasi* for (1, 2, 3, 4 and 5 days) was studied. And the effect on pathogenic properties of EPNs on *Galleria mellonella* was also studied. **Results:** It was found that, mortality percentage of EPNs depended on nano-Ag. Concentrations and the exposure time. There was a slight effect on pathogenicity, while there was a significant effect on EPNs reproductivity with the two concentrations (500 and 1500 ppm). **Conclusion:** Nanosilver can be used to control plant parasitic nematodes without excessive in order to don't harm the useful nematodes, EPNs.

Key words: Entomopathogenic nematodes, *Heterorhabditis indica*, *Steinernema arenarium*, *Steinernema abbasi*, silver nanoparticles, nano-Ag

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Nematodes have many advantages including simple and cheap productive cultures, a wide range of hosts and safety for the environment and higher organisms (Georgis and Gaugler, 1991).

Two families of entomopathogenic nematodes (EPNs): Steinernematidae and Heterorhabditidae play an important role in the biological control of insect pests. Nanotechnology is a branch of science that deals with production and application of materials, whose size is expressed in nanometers. The main aim of nanotechnology is to use nanomaterials and their biological properties in various scientific disciplines (Myczko, 2006). The EPNs found naturally in the soil environment and contribute to the insect pests control (Poinar, 1979). Insect larvae, which infected by the EPNs, die within 48 h (Georgis and Gaugler, 1991). Nanotechnology has a great impact on biological sciences such as agriculture (Georgis and Gaugler, 1991; Myczko, 2006). Silver nanoparticles are used in medical and agriculture (Myczko, 2006) as, such as nanocolloidal copper, which used in industry, agriculture and medicine. As active ingredients, AgNP possesses bactericidal and fungicidal effects and has been frequently used in the field of medicine (Wright *et al*., 1999; Yin *et al*., 1999; Furno *et al*., 2004; Kim *et al*., 2007). The AgNP has also 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). The properties of substances may be differs according to their form for example, in the ionic form silver might be toxic for organisms but silver nanoparticles have a wide spectrum of biological properties even at low concentrations (Williams, 2002; Myczko, 2006), these high biochemical reactivity may be due to the huge reactive surface of the nanoparticles (Myczko, 2006).

In this study, the effect of different concentrations of nano-Ag on EPNs viability up to 5 days and the pathogenic properties on *G. mellonella* was also studied.

MATERIALS AND METHODS

Multiplication and maintenance of the entomopathogenic nematodes: Nematodes in this investigation was represented by *Steinernema arenarium* (Sr), *Steinernema abbasi* (Sa) and *Heterorhabditis indica* (Hi). These nematodes were obtained from Department of Zoology and Agricultural Nematology, Faculty of Agriculture, Cairo University. The nematodes were propagated on the fifth instar larvae of the greater wax moth (*Galleria mellonella* L.), a lepidopteran host that is highly susceptible to parasitic infection. Insect larvae were placed in a 9 cm diameter petri dish lined with a moistened filter paper and exposed to about 100 IJ3s at 25°C. After 2 days, dead larvae (cadavers) were transferred to spongy trap dishes, which consisted of a wetted spongy trap consists of 8 cm length×4 cm width×5 mm thickness, which was placed in a petri dish and kept in a plastic bag. The amount of water in wetted sponge was calculated approximately as 10 mL of water/1 g of sponge, they all maintained at 25±2°C (Kassab and Entsar, 2016) After 10 days, all number of IJ3s was collected.

Rearing of the insect host (greater wax moth, *G. mellonella*): *Galleria mellonella* were obtained Plant Protection Department, Faculty of Agriculture, Ain Shams, Shoubra El-Kheima and reared on the artificial feeding media in transparent plastic jars at 28±2°C in the laboratory. Eggs that laid in masses were gently removed and incubated in other rearing jars provided with the hatching medium (Kassab and Entsar, 2016).

Preparing of silver nanoparticles (AgNP): Silver nanoparticle preparation: The AgNP used in this study was chemically produced (Fan *et al*., 2009). About 1500, 500, 200, 40 and 20 ppm of AgNP were used.

Experimental methods

Effect of different concentrations of AgNP on EPNs viability: The 100 IJ3s of EPNs were used for direct exposure assay to AgNP were added to another mL of solutions containing 1500, 500, 200, 40, 20 and 0 ppm of AgNP mg mL^{-1} of AgNP with five replications of each concentration. All replicates were incubated at 25±2°C. Nematode activity was measured at 1, 2, 3, 4 and 5 days after the AgNP treatments to determine the effective concentration, which affecting nematodes viability. Healthy nematodes were defined as those were curled whereas unhealthy nematodes were defined as those appeared straight-bodied. After 5 days of the AgNP treatment, the nematodes were washed with a sieve and resuspended in tap water. Healthy nematodes were counted after 2 h to determine if AgNP killed or temporary inactivated the movement of nematodes. Correct mortality was calculated.

Effect of different concentrations of AgNP on EPNs pathogenicity and reproductivity: One milliliter of the healthy nematodes of the previous experiments of each concentration were added separately to a 5th larval instar of

G. mellonella in a small petri dish lined with filter paper. One millilter of untreated EPNs IJ3s of EPNs was used as a control. All treatments were replicated 10 times. After 2 days, the insect mortality percentages were recorded, cadavers were washed and divided into two groups, the larvae of first were dissected and IJs were counted. While, the larvae of the second were transferred into the spongy trap.

RESULTS AND DISCUSSION

Effect of different concentrations of AgNP on EPNs viability: Nematodes mortality in solutions of silver nanoparticles (1500, 500, 200, 40 and 20 ppm) was estimated every day up to 5 days. The mortality of invasive larvae of EPNs exposed to nano-Ag depended on the concentration of nanoparticles and the time of exposure. The mortality of entomopathogenic nematodes increased with the increasing of concentration of nano-Ag and exposure time (Table 1), the susceptibility of EPNs varied according to the species of EPNs, the highest effect was found on Sa followed by Sr, while the lowest effect was recorded with Hi. The highest concentration of nanoparticles (1500 ppm) caused the highest percentage of mortality in Hi and Sr and Sa on the 5th day of experiment (11.5, 13.5 and 15.5%, respectively) and these data was agree with Kucharska and Pezowicz (2009) who found that, nano-Ag did not effect on the viability of Hb, while there was a slight effect on their ability of killing *Alphitobius diaperinus*. Kucharska *et al*. (2011a) found the same data on Hb and Sf and their pathogenicity against *G. mellonella*.

Effect of different concentrations of AgNP on EPNs pathogenicity and reproductivity: Pathogenicity of entomopathogenic nematodes that contacted to nano-Ag. concentrations did not differ significantly to their host *G. mellonella*.

In the case of Hi, mortality percentage of insects infected by Hi which survived 5 days long contact with all concentrations of AgNP was 100% similar to the control (except the final concentration reduced to be 98%), While it was decreased in Sr and Sa in the two last concentrations to be 93, 86.5 in Sr and 92, 79.4% in Sa, respectively (Fig. 1) and this is may be due to that, the nematode's symbiotic bacteria are immune to nano-Ag (Kucharska and Pezowicz, 2009; Kucharska *et al*., 2011b).

The same letters above bars have no significant differences, while different letters above bars indicate statistically significant differences in their effects.

In spite of the slight effect of nano-Ag on the insect mortality percentage, the initial population (Pi) the final population (pf) and the rate of reproduction (Rr) of EPNs was affected especially with the high concentrations as showed in Table 2. While, Kucharska *et al*. (2011a) reported a negative

Table 1: Effect of nano-Ag on mortality of the IJs of *H. indica*, *S. arenarium* and *S. abbasi*

Solution	After 1 day			After 2 days			After 3 days			After 4 days			After 5 days		
of AgNP	Hi	Sr	Sa	Hi	Sr	Sa	Hi	Sr	Sa	Hi	Sr	Sa	Hi	Sr	Sa
20 ppm	1.5±0.87^{b}	2.3±0.4^{b}	3.5±1.1^{c}	1.75±0.83^{b}	2.5±0.5^{c}	3.5±0.5^{d}	2.75±0.4^{b}	3.1±0.5^{d}	3.5±0.5^{c}	2.75±0.4^{b}	3.9±0.7^{c}	4.3±0.4^{c}	3.00±0.7^{b}	4.0±0.7^{d}	4.6±0.4^{d}
40 ppm	2.5±0.87^{b}	3.3±o.4^{b}	3.4±0.4^{c}	3.00±0.71^{b}	3.8±0.8^{c}	3.8±0.4^{cd}	3.75±0.4^{b}	4.1±0.9^{cd}	4.0±0.7^{bc}	3.75±0.8^{b}	4.3±0.4^{c}	4.8±0.4^{bc}	3.75±1.1^{b}	5.1±0.5^{cd}	5.8±0.4^{cd}
200 ppm	2.5±0.5^{b}	3.8±0.8^{b}	4.8±1.1^{c}	3.25±0.83^{b}	4.4±0.8^{c}	5.0±0.7^{c}	4.00±1.87^{b}	5.0±0.7^{c}	5.5±0.5^{b}	4.00±1.6^{b}	5.3±0.4^{c}	6.4±0.4^{b}	4.75±1.5^{b}	6.4±0.4^{c}	7.4±0.4^{c}
500 ppm	7.5±0.87^{a}	8.3±0.4^{a}	8.8±0.4^{b}	7.75±0.83^{a}	8.4±0.8^{b}	9.1±0.7^{b}	8.50±1.1^{a}	8.8±0.4^{b}	10.8±0.8^{a}	8.50±0.9^{a}	10.0±0.7^{b}	12.0±0.7^{a}	9.25±0.4^{a}	11.1±0.7^{b}	12.5±1.1^{b}
1500 ppm	8.5±1.66^{a}	9.5±0.5^{a}	10.8±0.43^{a}	9.25±1.3^{a}	10.8±0.8^{a}	11.4±0.41^{a}	9.75±1.5^{a}	11.1±0.5^{a}	12.1±0.8^{a}	10.25±1.8^{a}	12.3±0.8^{a}	13.0±1.2^{a}	11.5±1.5^{a}	13.5±0.5^{a}	15.5±1.1^{a}

Numbers followed by the same letters did not differ significantly in their effects while, different letters had a statistically significant differences

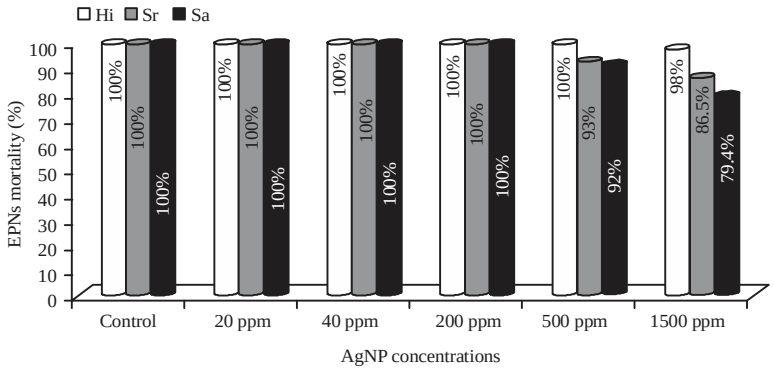

Fig. 1: Effect of nanoparticles on pathogenic properties of the nematodes *H. indica*, *S. arenarium* and *S. abbasi* exposed for 5 days to nano-Ag solutions

Table 2: Effect of nano-Ag on penetration and reproduction of *H. indica*, *S. arenarium* and *S. abbasi* on *G. mellonella*

	Hi			Sr			Sa		
	Pi	Pf×100	Rr	Pi	Pf×100	Rr	Pi	Pf×100	Rr
Control	29.3±2.5^{a}	1617±225^{a}	5518±409^{a}	21.7±1.2^{a}	323±25^{a}	1490±182^{ab}	27.7±0.5^{a}	190±48^{a}	685±169^{b}
20 ppm	20.0±1.6^{b}	1525±54^{a}	7625±835^{a}	17.7±1.2^{ab}	290±8^{a}	1638±139^{ab}	15.7±1.7^{b}	202±22^{a}	1286±105^{b}
40 ppm	19.0±0.8^{b}	1524±749^{a}	8022±64^{a}	15.0±2.4^{bc}	274±34^{a}	1828±175^{a}	12.3±1.7^{bc}	195±46^{a}	15830±213^{ab}
200 ppm	15.7±2.5^{bc}	1359±113^{a}	8657±1454^{a}	15.0±2.2^{bc}	258±16^{a}	1722±150^{a}	9.7±1.2^{cd}	268±31^{a}	2766±599^{a}
500 ppm	13.3±2.6^{bc}	8730±90^{b}	6566±1226^{a}	14.3±2.1^{bc}	129±9^{b}	9030±156^{b}	6.3±2.5^{d}	590±22^{b}	942±617^{b}
1500 ppm	10.7±0.8^{c}	6830±140^{b}	6386±2114^{a}	10.0±0.8^{c}	142±31^{b}	1417±424^{ab}	6.7±1.2^{d}	410±15^{b}	614±288^{b}

Numbers followed by the same letters did not differ significantly in their effects while, different letters had a statistically significant differences

effect of analysed solutions of nano-Au on pathogenic properties of *S. feltiae* on *A. diaperinus*, mortality of infection was 0% in this case.

CONCLUSION

The mortality of invasive larvae of heterorhabditis indica, Steinernema arenarium and *S. abbasi* exposed to nano-Ag depended on the concentration of nano silver and the exposure time.

Mortality of *Galleria mellonella* were different for nematodes that contacted with nano-Ag. But not significant. The reproductivity of EPNs within their hosts was affected significantly at the two highest concentrations from the control.

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