



# In Vitro Effectiveness of Silver Nanoparticles against Root-Knot Nematode (*Meloidogyne incognita*)

Khansa Nazir<sup>1,\*</sup>, Tariq Mukhtar<sup>1</sup> and Humayun Javed<sup>2</sup>

<sup>1</sup>Department of Plant Pathology, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi

<sup>2</sup>Department of Entomology, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi

## ABSTRACT

As the use of nematicides is becoming prohibitive in many countries due to their detrimental consequences, the management of plant parasitic nematodes using nanoparticles can be one of the important alternatives. In the present study, the nematicidal activity of silver nanoparticles (AgNP) was investigated against the most destructive root-knot nematode (*Meloidogyne incognita*). The maximum mortality of juveniles was recorded at a concentration of 100 mg/ml followed by 75 mg/ml of AgNP. The minimum mortality was recorded with 25 mg/ml of AgNP. With the increase in concentration, there was a corresponding increase in the mortality of juveniles showing a direct relationship between mortality and concentration of nanoparticles. The effect of time on mortality was also found significant. With the increase in time, there was a corresponding increase in mortality and the relationship was found to be directly proportional. Similarly, the maximum hatching inhibition of *M. incognita* eggs was recorded at a concentration of 100 mg/ml of nanoparticles followed by 75 mg/ml of AgNP. The minimum inhibition was recorded with 25 mg/ml of AgNP. It was found that with the an increase in concentration, there was a corresponding increase in hatching inhibition showing direct relationship between hatching inhibition and concentrations of the nanoparticles. Similarly, the maximum hatching inhibitions were recorded after 6<sup>th</sup> day followed by 5<sup>th</sup> day. The hatching inhibition was found directly proportional to time duration as it increased with an increase in number of days. It is concluded from the present study that AgNP possess nematicidal activity against root-knot nematodes and can act as an alternative to high-risk synthetic nematicides or inconsistent biological control agents without causing any phytotoxicity.

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## Authors' Contribution

KN and TM designed the study, conducted the surveys, executed experimental work, analyzed the data and prepared the manuscript. HJ provided technical assistance. TM supervised the work.

## Key words

Root-knot nematodes, Silver nanoparticles, Hatchability, Mortality, Toxicity.

## INTRODUCTION

Many pests including plant pathogenic nematodes attack vegetables and a number of crops and are responsible for causing severe growth retardations (Ashfaq *et al.*, 2017; Fateh *et al.*, 2017; Ifthikhar *et al.*, 2018; Javed *et al.*, 2017a, b; Kassi *et al.*, 2018, 2019a, b; Nabeel *et al.*, 2018; Aslam *et al.*, 2019a). Among plant parasitic nematodes, root-knot nematode (*Meloidogyne incognita*), is one of the most important nematodes associated with low production of many vegetables in Pakistan (Kayani *et al.*, 2017; Kayani and Mukhtar, 2018; Tariq-Khan *et al.*, 2017). Root-knot nematodes are ranked at the top among the five major plant pathogens and the first among the ten most important genera of plant parasitic nematodes in the world (Mukhtar *et al.*, 2017a; 2018). They have wide geographic distribution, large host range and high destructive potential. They have been reported to be implicated with other plant pathogens like *Ralstonia solanacearum* and result in

disease complexes and aggravation of wilt diseases (Aslam *et al.*, 2017a, b, 2019b). On the other hand, biological control agents can cause significant reductions in the soil populations of these nematodes (Khan *et al.*, 2017; Mukhtar *et al.*, 2017b; Rahoo *et al.*, 2017, 2018a, b, 2019).

In Pakistan, *M. incognita* has been found one of the most dominant root-knot species and rampant in the vegetable-producing areas of Pakistan and considerably reduces growth and yield (Kayani *et al.*, 2018). The worldwide distribution of this species is 47% and in Pakistan its overall occurrence is 52%. Overall yield losses of 50 to 80% have been reported to be caused by root-knot nematodes in vegetables and 24 to 38% yield losses due to root-knot nematodes have been estimated on tomato. Root-knot nematodes have become a serious threat to the profitable cultivation of vegetables in the country. The yield losses by root-knot nematodes are mainly caused due to buildup of inoculum of the nematode and repeated cultivation of same cultivars in the same land every year (Hussain *et al.*, 2016, 2019).

Root-knot nematodes are mainly controlled by the application of nematicides and resistant cultivars. Although nematicides can effectively manage nematodes

\* Corresponding author: [khansanazir.65@gmail.com](mailto:khansanazir.65@gmail.com)  
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but their usage is limited due to their short-term effects, high costs, non-availability, resistance development in nematodes, health and environmental hazards, residual toxicity and adverse effects on the beneficial micro flora and fauna in the soil besides phytotoxic effects on the crop.

To overcome the deleterious effects of nematicides, alternative strategies are being sought. Nanotechnology has the potential to revolutionize the agricultural and food industry with new tools for the molecular treatment of diseases, rapid disease detection, enhancing the ability of plants to absorb nutrients and also smart sensors and smart delivery systems which will help the agricultural industry combat viruses and other crop pathogens (Joseph and Morrison, 2006). For the last few years, nanoparticles have been encouraged to control plant pathogens including nematodes (Lara *et al.*, 2011). Nanotechnology is mainly concerned with the production and utilization of materials whose dimensions are expressed in nanometers (1-100 nm or  $1.5 \times 10^9$  m). The nanoparticles (NP) have a high capacity percentage in terms of volume to surface that enhances their affectivity and expected biochemical properties (Dubchak *et al.*, 2010). Similarly, silver nanoparticles (AgNP) can be applied as sensors, catalysts, anticancer agents and antimicrobial agents as they are the advanced type of material. AgNP have shown response against different kinds of pathogens. In the present study, the nematocidal activity of silver nanoparticles was investigated against the most destructive root-knot nematode (*Meloidogyne incognita*).

## MATERIALS AND METHODS

### Preparation of silver nanoparticles

Silver nanoparticles (AgNP) were prepared by using onion extract as reducing agent and by the chemical decay of silver nitrate. For the extraction of onion, 25 g onion was taken and cut down into small pieces. In a pan, 300 ml distilled water was taken and boiled for 15 to 20 min under medium flame. After appearance of the bubbles, onion was added into the water until the solution color changed to light green. At the end, the solution was sieved and cooled. Silver nitrate ( $\text{AgNO}_3$ ) solution was made by dissolving 315 mg silver nitrate in 1 liter of distilled water and boiled for 2 to 3 min in an oven. The solution was condensed stepwise by adding 5 ml onion extract. The solution was again boiled for one minute and maintained in light for 2 to 3 min until the solution color changed. In this way 200 ppm concentration of AgNP solution was obtained and used for further applications.

### Nematode culture

An indigenous population of root-knot nematode

(*Meloidogyne incognita*) initially isolated from cucumber roots, identified on the basis of perineal pattern and maintained on the highly susceptible cultivar of tomato (money maker) was used in the assessment. The nematode was mass produced on tomato cv. Money maker (Mukhtar *et al.*, 2017b).

### Collection of juveniles and egg masses

For collection of juveniles, eggs were extracted using sodium hypochlorite (Hussey and Barker, 1973). The eggs were then processed on extraction trays for emergence of second stage juveniles (Whitehead and Hemming, 1965). The freshly hatched juveniles were collected in a beaker, standardized and concentrated. *M. incognita* infected tomato plants were uprooted and gently washed under running tap water. Uniform-sized egg masses were collected using forceps and the eggs per egg mass were counted (Mukhtar *et al.*, 2013).

### Effects of AgNP on juvenile mortality and egg hatching

To assess the effects of silver nanoparticles, 5 ml of solutions containing 0, 25, 50, 75 and 100 mg/ml of AgNP were added to 2.5 cm diameter plastic Petri plates. Approximately 30 juveniles of *M. incognita* contained in 50  $\mu\text{l}$  were added to each solution. There were five replications for each concentration. The plates were incubated at room temperature in a completely randomized design. The mortality of juveniles was recorded after 12, 24, 48 and 72 h. Nematodes were considered alive if they moved or appeared as a winding shape and were considered dead if they did not move when probed with a fine needle (Mukhtar *et al.*, 2013).

To evaluate the effects of solutions on hatchability, 5 ml of solutions containing 0, 25, 50, 75 and 100 mg/ml of AgNP was added to 2.5 cm diameter plastic Petri plates. One uniform-sized egg mass containing approximately 200 eggs was added to each solution. There were five replications for each concentration. The plates were incubated at room temperature in a completely randomized design. The hatching of juveniles was recorded daily up to the 6<sup>th</sup> day under stereomicroscope. After each count, the egg masses were washed with 1 ml of sterilized distilled water in their respective plates and transferred to fresh solutions of the same concentration. The percent mortality and hatching inhibition in each treatment was calculated and corrected by Abbott's formula (Abbott, 1925) as follows:

$$\text{Mortality or HI (\%)} = \frac{t - c}{100 - c} \times 100$$

Where, HI is hatching inhibition, t is percent mortality or hatching inhibition in the extract and c is percent mortality or hatching inhibition in the control.

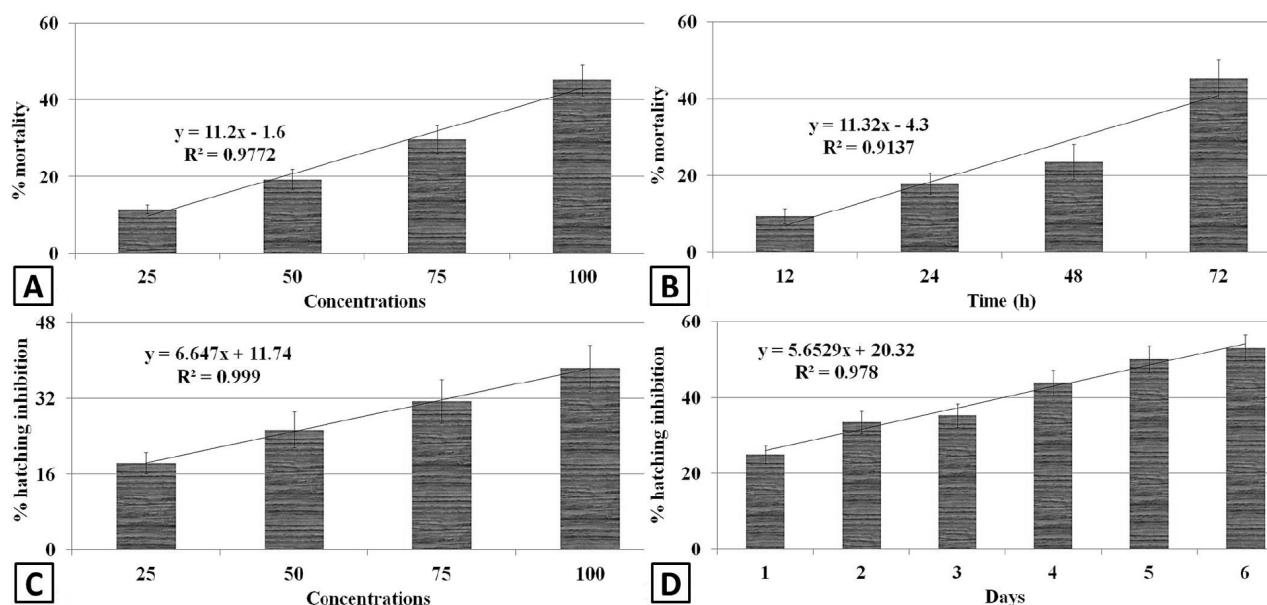


Fig. 1. Effect of concentrations of silver nanoparticles (A), and time (B) on mortality of *Meloidogyne incognita*. Effect of concentrations of silver nanoparticles (C), and days (D) on hatching inhibition of *Meloidogyne incognita*.

#### Statistical analysis

The data were subjected to analysis of variance using GenStat package 2009 (12<sup>th</sup> edition) version 12.1.0.3278 (www.vsni.co.uk) and the means were compared by Fisher's Protected Least Significant Difference Test. A significance level of  $p \leq 0.05$  was used in statistical analyses. The linear relationships between concentrations and time durations as independent variables (x) and mortality and hatching inhibitions as dependent variables (y) were calculated in Microsoft Excel 2007. Standard errors of means were also calculated in Microsoft Excel 2007.

## RESULTS

#### Effect of silver nanoparticles on mortality

The analysis of variance regarding effects of nanoparticles on mortality of *M. incognita* showed highly significant results. The effects of time and concentrations were also found significant. Maximum mortality of juveniles was recorded at a concentration of 100 mg/ml followed by 75 mg/ml of AgNP. The minimum mortality was recorded with 25 mg/ml of AgNP. It was also observed that with the increase in concentration there was a corresponding increase in the mortality of juveniles showing a direct relationship between mortality and concentrations of the nanoparticles as shown in Figure 1A. The effect of time on mortality was also found significant. Maximum mortality was recorded after 78 h and the minimum mortality was observed after 12 h. It was

also observed that with the increase in time, there was a corresponding increase in mortality and the relationship was found directly proportional as shown in Figure 1B.

#### Effect of nanoparticles on hatching inhibition

The Analysis of Variance regarding effect of silver nanoparticles on hatching inhibition of *M. incognita* showed highly significant results. The effects of days and concentrations were also found significant. Maximum hatching inhibition of *M. incognita* eggs was recorded at a concentration of 100 mg/ml of nanoparticles followed by 75 mg/ml of AgNP. The minimum inhibition was recorded with 25 mg/ml of AgNP. It was found that with an increase in concentration, there was a corresponding increase in hatching inhibition showing direct relationship between hatching inhibition and concentrations of the nanoparticles as shown in the Figure 1C. Similarly, days also significantly affected hatching inhibition. Maximum hatching inhibitions were recorded after 6<sup>th</sup> day followed by 5<sup>th</sup> day. The relationship between hatching inhibition and days was found to be direct as hatching inhibition increased with an increase in number of days as shown in Figure 1D.

## DISCUSSION

In the present study, AgNP significantly caused mortality and hatching inhibition of *M. incognita* under lab conditions. Earlier a number of workers reported the

toxicity of nanoparticles against root-knot nematodes. Taha and Abo-Shady (2016) assessed the nematicidal effects of AgNP against *M. incognita* juveniles in the laboratory and achieved 96.5 percent mortality after 72 h with 1500 ppm. The concentration of 200 ppm caused 52 percent mortality on the third day and 1500 ppm was found to be the most effective dose while other doses were ineffective. Ardakani *et al.* (2013) evaluated the efficacy of AgNP on *M. incognita* infecting tomato and found the doses of 800, 400 and 200 mg/ml of AgNP highly effective in causing immobility and mortality of *M. incognita* juveniles. Abdellatif *et al.* (2016) studied the influence of AgNP on the reproduction and growth of *M. javanica* and reported that AgNP treatment was equally effective as chemical treatment (Vydate 24% L) resulting in a reduction of egg masses per root system. AgNP were also found effective in improving growth of plants. There are reports that AgNP enhanced seed germination, seedling vigor and increased shoot length, root length, number of leaves and other plant growth criteria of different crops (Pandey *et al.*, 2014; Yasmeen *et al.*, 2015; Hojjat, 2016; Zuverza-Mena *et al.*, 2016).

The nematicidal effect of AgNP is not specific and can be equally effective against other plant parasitic nematodes and plant pathogenic fungi in addition to root-knot nematodes. AgNP have been found 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 and Salopek-Sondi, 2004; Morones *et al.*, 2005; Lok *et al.*, 2006; Choi and Hu, 2008). This is why, AgNP have shown a broad-spectrum antimicrobial activity and can affect plant pathogenic bacteria and fungi (Park *et al.*, 2006; Jo *et al.*, 2009). As such, it is possible that AgNP displays an antifungal effect on common root-associated fungal pathogens like *Gaeumannomyces graminis* and *Rhizoctonia solani*, in warm-season turfgrass. Similarly, turfgrass treated with AgNP can become more tolerant to root-knot nematode damage because of its protection from additional weakness and stress brought on by these other pathogens. Therefore, AgNP may provide an additional benefit of managing multiple turfgrass pathogens and contribute to turf quality improvement (Cromwell *et al.*, 2014). As high frequency and application doses of AgNP are needed for effective control of root-knot nematodes, therefore, their application as fertigation and combination with compatible chemicals may enhance the applicability of AgNP. Further understanding of the mechanism in the nematicidal action of AgNP also warrants improvement of AgNP efficacy.

## CONCLUSION

It is concluded from the present study that AgNP possess nematicidal activity against root-knot nematodes and can act as an alternative to high-risk synthetic nematicides or inconsistent biological control agents without causing any phytotoxicity.

### Statement of conflict of interest

The authors declare no conflict of interest.

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