# *In vivo* evaluation of silver nanoparticles on the management of *Meloidogyneincognita* in green gram


## ABSTRACT

In the investigations, on the effect of AgNPs on the management of *M. incognita* in green gram, the results showed that all the treatments were found to be effective in increasing all growth parameters. Maximum growth parameter *viz.*, plant height, fresh and dry weight of shoot and root were recorded in the treatment with 0.15 ppm AgNPs + *M. incognita*. Maximum reduction in galls, eggmasses and final nematode population in soil were recorded in the treatment with 0.15 ppm AgNPs. All the treatments significantly differed from control except the treatment with 0.0015 ppm AgNPs + *M. incognita.*



**Keywords:**Silver nanoparticles (AgNPs), *Meloidogyne incognita,* green gram


## INTRODUCTION

Green gram [*Vigna radiata* (L.) Wilzek] is one of the most important pulse crop grown throughout the country. The crop green gram suffers from a number of diseases caused by fungi, bacteria, virus and nematode. Among them, the plant parasitic nematodes are considered to be one of the most important groups and eventually affect the yield of the crop. In mung bean (*Vigna radiata*) avoidable losses due *M. javanica* under field condition ranged from 42.1 to 93.4 per cent (Gupta and Verma, 1990). This nematode also interferes with nitrogen fixation thereby contributing future loss in yields of pulse crops.

"Nanotechnology is a new emerging and fascinating field of science. It is the science largely dealing with synthesis and application of nanoparticles.

Nanoparticles (nano-scale particles = NSPs) are atomic or molecular aggregates with at least one dimension between 1 and 100 nm" (Ball, 2002)."Silver nanoparticles (AgNPs) have broad spectrum antimicrobial or biocidal properties against all classes of microorganisms and possess numerous distinctive physio-chemical properties compared to bulk Ag. Hence, AgNPs are among the most widely used engineered NPs in a wide range of consumer products and are expected to enter natural ecosystems including soil via diverse pathways" (Anjum *et al.,* 2013).

"Though recent nanomaterials and nanotechnology have been widely applied in the world in this last decade, Nanotechnology provides the tools and the technological platforms for the study and transformation of biological systems. Few studies have focused on the effects and mechanisms of nano-materials on plants. Studies have shown that NPs may undergo accumulation in plants. Since plants possess a large size and high leaf area, and are of stationary in nature they have a greater chance of exposure to a wide range of NPs available in their surrounding environment" (Dietz and Herth, 2011). Thesehigher plants strongly interact with their atmospheric and terrestrial environments and are expected to be affected as a result of their exposure to NSPs.

"Development of reliable and eco-friendly process for silver nanoparticles is an important step in the field of application of nanotechnology. However, it is only recently that microorganisms have been explored as potential bio factory for synthesis of metallic nanoparticles such as cadmium sulphide, gold and silver. Ag is considered to be one of the most toxic trace metals, and the widespread use of AgNPs has increased the likelihood of releases into the environment, which underscores the need to assess the impacts of AgNPs on the ecosystem's health". (Ratte, 1999)AgNPs are reported to be potentially toxic to bacteria (Morones *et* *al*., 2005), algae (Miao *et al*., 2010), human cells (Jiang *et al*., 2008; AshaRani *et al*., 2009), and animal cells (Hussain *et al*., 2005). “AgNPs increased plants growth profile (shoot and root length, leaf area) and biochemical attributes (chlorophyll, carbohydrate and protein contents, antioxidant enzymes) of *Brassica juncea*, common bean and corn” (Salama, 2012). Syu *et al*. (2014) observed that “AgNPs showed the highest degree of root growth promotion (RGP)”. Pankaj *et al.* (2012) reported that “application of silver nanoparticles were effective in increasing shoot length and CR formulations (CP1 and CP2) significantly reduced the number of galls, when compared to CP0 in tomato plant”.

Cormwell *et al.* (2014) reported that“application of AgNPs improved turfgrass quality in one year and reduced gall formation in the roots in bermudagrass”.

Although the root-knot nematode is a recognized pest of leguminous crop in Assam, no work so far has been done in the management of root-knot nematode associated with green gram by use of silver nanoparticles (AgNPs). Therefore present investigation is an attempt to study the efficacy of nanoparticles on the management of root knot nematode, *M. incognita* in green gram.

## MATERIALS AND METHODS

The experiment was carried out in the net house of Department of Nematology during *rabi* season 2016-2017 to study the effect of silver nanoparticles on the management of *M. incognita* in green gram. Silver nanoparticles (AgNPs) treated seeds of green gram (var. Pratap) were sown in 1kg capacity pot filled with sterilized soil. After germination thinning was done and kept one healthy seedling in each pot. Ten days old plants were inoculated with 1000 freshly hatched second stage juvenile of *M. incognita* either singly or in combination by removing small quantity of soil around the root system, followed by inoculation and replacement of removed soil. There were 7 treatments and treatments were as follows:

**Treatments**

| | | |
|---|---|---|
| $T_1$ | : | Control (UC) |
| $T_2$ | : | Control (Nematode alone) |
| $T_3$ | : | 0.0015 ppm AgNP + *Meloidogyne incognita* |
| $T_4$ | : | 0.003 ppm AgNP+ *M. incognita* |
| $T_5$ | : | 0.015 ppm AgNP + *M. incognita* |
| $T_6$ | : | 0.03 ppm AgNP + *M. incognita* |
| $T_7$ | : | 0.15 ppm AgNP + *M. incognita* |

Each treatment was replicated for 5 times. Regular watering was done till the harvesting of the crop. Insect pests when appeared were killed manually. For observations on different parameters, plants of each treatment were uprooted carefully at 60 days after the inoculation of nematode. The entire root system was taken out from the pot and kept in a plastic bucket half filled with water for half an hour and then washed carefully with tap water. Observations were made on shoot length, number of galls, eggmasses per root system, fresh and dry weight of shoot and root, final nematode population in soil and number of root nodules per root system.

“For recording the dry weight of shoots and roots were separately cut in to small pieces and kept in an oven running constantly at 60°C. The materials were weighted at every 24 hours interval till a constant weight was obtained. For recording the final nematode population at first side the entire amount of soil from each pot was mixed homogenously and drawn 250 cc of it and processed by following Cobb's modified sieving technique" (Christie and Perry, 1951).

## RESULT AND DISCUSSION

Studies on *in vivo* evaluation of silver nanoparticles on the management of *M. incognita* in green gram revealed that the maximum increase in plant growth parameters *viz*., shoot length (49.66 cm), fresh weight of shoot (31.06 g), dry weight of shoot (6.54 g) were recorded in the treatment with 0.15 ppm AgNPs + *M. incognita* @ 1000 $J_2$/kg of soil. Similar findings were observed by Pankaj *et al.* (2012), who reported that "application of silver nanoparticles were effective in increasing shoot lenth". Hasan *et al*. (2016) reported that "the growth parameters showed significant increase in shoot length which reached to (40 cm) in the combination of oxamyl and nanoparticles compared under greenhouse conditions".

All these treatments brought about a significant decreased in numbers of root galls, egg masses and nematode population in soil over control. The minimum numbers of galls and eggmasses were recorded in the treatment with 0.15 ppm AgNPs + *M. incognita* @ 1000 $J_2$/kg of soil. All the treatments differed significantly from control. Similar findings were observed by Pankaj *et al.* (2012) reported that "application of silver nanoparticles significantly reduced in root galls in tomato". Cormwell *et al.* (2014) reported that "biweekly application of 90.4 $mg/m^2$ of AgNPs improved turfgrass quality in one year and reduced gall formation in the roots in bermudagrass". Taha *et al.* (2016) found that "all the concentrations of AgNPs inhibited the nematode growth, galls, egg formation, final population and eggs hatchability in tomato plant. They recorded that the high concentrations of 200 ppm, 500 ppm and 1500 ppm were more significant in their effect. Sharaf *et al*. (2016) also found that application of silver nanoparticles (AgNPs) against root-knot nematode *M. incognita* infecting tomato plants resulted in 86% and 88% reduction of root galls and eggmasses respectively”.

Abdellatif *et al*. (2016) also found that “Green Synthesis Nanoparticle (17 mg $mL^{-1}$) obtained from *U. lactuca* was more effective in reducing second-stage juveniles (J2s) of *M. javanica* (69.44%) population in soil”.

Nazir *et al.* (2019) studied “the *in-vitro* effectiveness of silver nanoparticles against root-knot nematode *Meloidogyne incognita* and the study revealed that 100 mg/ml AgNP caused the maximum mortality of nematodes and maximum inhibition of egg hatching. It was observed that the concentration of AgNPs and time of exposure were indirectly proportional to mortality and egg hatching”**.**

Kalaiselvi *et al.* (2019) worked on green synthesis of silver nanoparticles from *Euphorbia tirucalli* for its use as a control measure against root-knot nematode *Meloidogyne incognita* and found significantly reduced infestation of the nematode in the plant roots as a result of root dip treatment with Et-AgNPs. Further, they observed that plant treatment with Et-AgNPs resulted in healthier plant growth parameters with reduced overall gall formation, egg masses and number of eggs per egg mass and $J_2$ population in soil. Hamed *et al*. (2019) biosynthesized silver nanoparticles from the Cyanobacterium *Nostoc* sp. PCC7524 for their evaluation of nematicidal activity against *Meloidogyne javanica* in faba bean and it was found that *in-vitro* assay against the root-knot nematode *Meloidogyne javanica* showed AgNPs significantly decreased egg hatching of *M. javanica* at different applied concentrations (3, 6, 12, 25, and 50%, v/v). The highest reduction percent (94.66%) was observed in 50% concentration of silver nanoparticles. Silver nanoparticles at a concentration of 2.4 ml/l for a period of 24 hours completely inhibited the growth of *Meloidogyne javanica* $J_2$ compared to 23% inhibition using aqueous cyanobacterial extract (ACE). Soil treatment with 3 different concentrations of AgNps (1, 2 and 3 ml/kg soil) resulted in significantly reduced gall formation and final $J_2$ population in soil over a course of 2 seasons. Further it was observed that 3 ml/kg soil of silver nanoparticles was the most effective concentration with considerably better growth of faba bean plants.

Baronia *et al.* (2020) conducted a study to assess the potential of silver nanoparticles as an effective nematicide against *Meloidogyne graminicola* and found 0.1 µg/ml as the minimum concentration for 100% irreversible nematode mortality after a period of 12 hours. In a sand screening test under the experiment, it was observed that a concentration of 2 µg/ml of AgNPs showed 100% nematicidal effect after an incubation period of 24 hours. The effective concentration of AgNPs for nematode control under field conditions was found to be 3 µg/ml.

Li *et al.* (2021) compared "the symptom and gall development in the susceptible cucumber inbred line Q24 and the resistant *Cucumis metuliferus* (CM) in response to *M. incognita.* The results showed that in comparison with Q24, the *Cucumis metuliferus* (CM) was able to significantly reduce penetration numbers of second stage juveniles ($J_2$), slow its development in the roots resulting in fewer galls and smaller giant cells suggesting the presence of host resistance in *Cucumis metuliferus* (CM). In the susceptible Q24, larger galls were observed throughout the root system".

Ghareeb *et al.*(2022) tested "the nematicidal activity of extracts of two marine algae(*Colpomenia sinuosa* and *Corallina mediterranea*) and their synthesized silver nanoparticles against root knot nematode infecting tomato plants and found that *C.*

*sinuosa* metheylene chloride extracts was more active than *C. mediterranea*. The most effective eluent of this solvent was hexane: Metheylene chloride: Ethyl acetate. In contrast to the other treatments C. sinuosa nanoparticles reduce the number of nematode galls, egg masses per root, and eggs/egg mass, while also improving plant growth parameters. The silver nanoparticles synthesized from C. sinuosa could be used as alternative chemical nematicides".

## Conclusion

Studies on I*n vivo* evaluation of silver nanoparticles on the management of *M. incognita* showed that the treatment with 0.15 ppm AgNPs was found to be best in increasing the plant growth parameter *viz.*, shoot length, fresh and dry weight of shoot and root, numbers of nodules and reducing the numbers of galls, egg masses and final nematode population in soil.

Nanotechnology approaches are required to develop new capabilities for prevention or treatment of plant pathogenic nematodes using nanoparticles, rapid detection of pest, and pathogen using nano-based kits.

**Table 1. Effect of AgNPs on plant growth parameters of green gram**

| Treatments | Shoot length (cm) | Fresh weight of shoot (g) | Dry weight of shoot (g) | Fresh weight of root (g) | Dry weight of root (g) | Numbers of nodules |
|---|---|---|---|---|---|---|
| $T_1$ : Control (UC) | 38.30$^d$ | 20.88$^d$ | 3.34$^d$ | 3.30$^d$ | 1.34$^d$ | 5.40$^d$ |
| $T_2$ : Control (Nematode alone) | 27.36$^f$ | 14.02$^f$ | 1.26$^f$ | 1.86$^f$ | 0.32$^f$ | 1.80$^f$ |
| $T_3$ : 0.0015 ppm + *Meloidogyne incognita* | 29.10$^f$ | 15.60$^f$ | 1.66$^f$ | 1.94$^f$ | 0.48$^f$ | 2.00$^f$ |
| $T_4$ : 0.003 ppm + *M. incognita* | 33.84$^e$ | 18.60$^e$ | 2.36$^e$ | 2.54$^e$ | 0.96$^e$ | 3.60$^e$ |
| $T_5$ : 0.015 ppm + *M. incognita* | 42.98$^c$ | 24.12$^c$ | 4.08$^c$ | 3.92$^c$ | 1.76$^c$ | 7.20$^c$ |
| $T_6$ : 0.03 ppm + *M. incognita* | 46.56$^b$ | 27.46$^b$ | 5.24$^b$ | 4.84$^b$ | 2.16$^b$ | 8.80$^b$ |
| $T_7$ : 0.15 ppm + *M. incognita* | 49.66$^a$ | 31.06$^a$ | 6.54$^a$ | 5.76$^a$ | 2.78$^a$ | 11.8$^a$ |
| S. Ed. (±) | 1.39 | 0.96 | 0.26 | 0.27 | 0.15 | 0.71 |
| CD (0.05) | 2.85 | 1.98 | 0.31 | 0.55 | 0.51 | 1.46 |

Means followed by the same letter shown in subscript (s) are not differed significantly

**Table 2. Effect of AgNPs on the development of *M. incognita* in green gram**

| Treatments | No. of galls | No. of egg masses | Final nematode population/250cc soil | % decreased over control |
|---|---|---|---|---|
| $T_1$ : Control (UC) | 0.00 | 0.00 | 0.00 | - |
| | $(0.71)^g$ | $(0.71)^g$ | $(0.71)^g$ | |
| $T_2$ : Control ( Nematode alone ) | 116.80 | 46.60 | 501.8 | - |
| | $(10.74)^a$ | $(6.80)^a$ | $(22.36)^a$ | |
| $T_3$ :0.0015 ppm + *Meloidogyne incognita* | 91.00 | 35.60 | 415.2 | 17.25 |
| | $(9.48)^b$ | $(5.98)^b$ | $(20.30)^b$ | |
| $T_4$ : 0.003 ppm + *M. incognita* | 46.40 | 17.40 | 319.0 | 36.42 |
| | $(6.78)^c$ | $( 4.02)^c$ | $(17.70)^c$ | |
| $T_5$ : 0.015 ppm + *M. incognita* | 27.20 | 13.20 | 251.0 | 49.98 |
| | $(5.20)^d$ | $(3.64)^d$ | $(15.80)^d$ | |
| $T_6$ : 0.03 ppm + *M. incognita* | 15.60 | 9.80 | 228.8 | 54.40 |
| | $(3.96)^e$ | $(3.14)^e$ | $(15.10)^e$ | |
| $T_7$ : 0.15 ppm + *M. incognita* | 7.00 $(2.68)^f$ | 5.80 $(2.46)^f$ | 206.2 $(14.38)^f$ | 58.90 |
| S. Ed. (±) | 0.34 | 0.16 | 0.25 | - |
| $CD_{0.05}$ | 0.70 | 0.34 | 0.51 | - |

Values of number of galls, eggmasses and final nematode population within parentheses are square root ( $\sqrt{x + 0.5}$ ) transformed data

Mean followed by the same letter in the superscript(s) are not significantly different


**Plate 1. General view of the pot experiment**

$T_1$ $T_2$ $T_3$ $T_4$ $T_5$ $T_6$ $T_7$

**Plate 2.Effect of silver nanoparticles on plant growth parameters of green gram**

$T_1$

$T_2$

$T_3$

$T_4$

$T_5$

$T_6$

$T_7$

**Plate 3. Effect of different treatments on root growth of green gram**

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