

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

### **ABSTRACT**

In [this](#) investigation, on the effect of AgNPs on the management of *M. incognita* in greengram, 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, egg masses 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). These higher 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 (Ratte, 1999), 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. 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*

**Comment [H1]:** Reference this please

*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). Syuet *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.

**Comment [H2]:** Write in full at point of first mention with the abbreviation in bracket please

Cormwellet *al.* (2014) reported that application of AgNPs improved turfgrass quality in one year and reduced gall formation in the roots in bermudaBermuda grass.

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 1\_kg 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:

**Comment [H3]:** Check for conflict of interest since the research was not published immediately

### Treatments

- T<sub>1</sub> : Control (UC)
- T<sub>2</sub> : Control (Nematode alone)
- T<sub>3</sub> : 0.0015 ppm AgNP + *Meloidogyne incognita*
- T<sub>4</sub> : 0.003 ppm AgNP+ *M. incognita*
- T<sub>5</sub> : 0.015 ppm AgNP + *M. incognita*
- T<sub>6</sub> : 0.03 ppm AgNP + *M. incognita*
- T<sub>7</sub> : 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, egg masses 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).

**Comment [H4]:** The word "Sacrificed" should be used instead. Researchers are not killers

**Comment [H5]:** Thereference is too old

## RESULTS 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<sub>2</sub>/kg of soil. Similar findings were observed by Pankaj *et al.* (2012), who reported that application of silver nanoparticles were effective in increasing shoot length. 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 decrease in numbers of root galls, egg masses and nematode population in soil over control. The minimum numbers of galls and egg masses were recorded in the treatment with 0.15 ppm AgNPs + *M. incognita* @ 1000 J<sub>2</sub>/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. Cornwell *et al.* (2014) reported that biweekly application of 90.4 mg/m<sup>2</sup> of AgNPs improved turfgrass quality in one year and reduced gall formation in the roots in bermuda grass. 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 egg masses respectively. Abdellatif *et al.* (2016) also found that Green Synthesis Nanoparticle (17 mg mL<sup>-1</sup>) obtained from *U. lactuca* was more effective in reducing second-stage juveniles (J2s) of *M. javanica* (69.44%) population in soil.

**Comment [H6]:** Can you give a plausible reason for this observation?

Studies on *In 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.

UNDER PEER REVIEW

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

<b>Treatments</b>	<b>Shoot length (cm)</b>	<b>Fresh weight of shoot (g)</b>	<b>Dry weight of shoot (g)</b>	<b>Fresh weight of root (g)</b>	<b>Dry weight of root (g)</b>	<b>Numbers of nodules</b>
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T <sub>1</sub> : Control (UC)	38.30 <sup>d</sup>	20.88 <sup>d</sup>	3.34 <sup>d</sup>	3.30 <sup>d</sup>	1.34 <sup>d</sup>	5.40 <sup>d</sup>
T <sub>2</sub> : Control (Nematode alone)	27.36 <sup>f</sup>	14.02 <sup>f</sup>	1.26 <sup>f</sup>	1.86 <sup>f</sup>	0.32 <sup>f</sup>	1.80 <sup>f</sup>
T <sub>3</sub> : 0.0015 ppm + <i>Meloidogyne incognita</i>	29.10 <sup>f</sup>	15.60 <sup>f</sup>	1.66 <sup>f</sup>	1.94 <sup>f</sup>	0.48 <sup>f</sup>	2.00 <sup>f</sup>
T <sub>4</sub> : 0.003 ppm + <i>M. incognita</i>	33.84 <sup>e</sup>	18.60 <sup>e</sup>	2.36 <sup>e</sup>	2.54 <sup>e</sup>	0.96 <sup>e</sup>	3.60 <sup>e</sup>
T <sub>5</sub> : 0.015 ppm + <i>M. incognita</i>	42.98 <sup>c</sup>	24.12 <sup>c</sup>	4.08 <sup>c</sup>	3.92 <sup>c</sup>	1.76 <sup>c</sup>	7.20 <sup>c</sup>
T <sub>6</sub> : 0.03 ppm + <i>M. incognita</i>	46.56 <sup>b</sup>	27.46 <sup>b</sup>	5.24 <sup>b</sup>	4.84 <sup>b</sup>	2.16 <sup>b</sup>	8.80 <sup>b</sup>
T <sub>7</sub> : 0.15 ppm + <i>M. incognita</i>	49.66 <sup>a</sup>	31.06 <sup>a</sup>	6.54 <sup>a</sup>	5.76 <sup>a</sup>	2.78 <sup>a</sup>	11.8 <sup>a</sup>
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

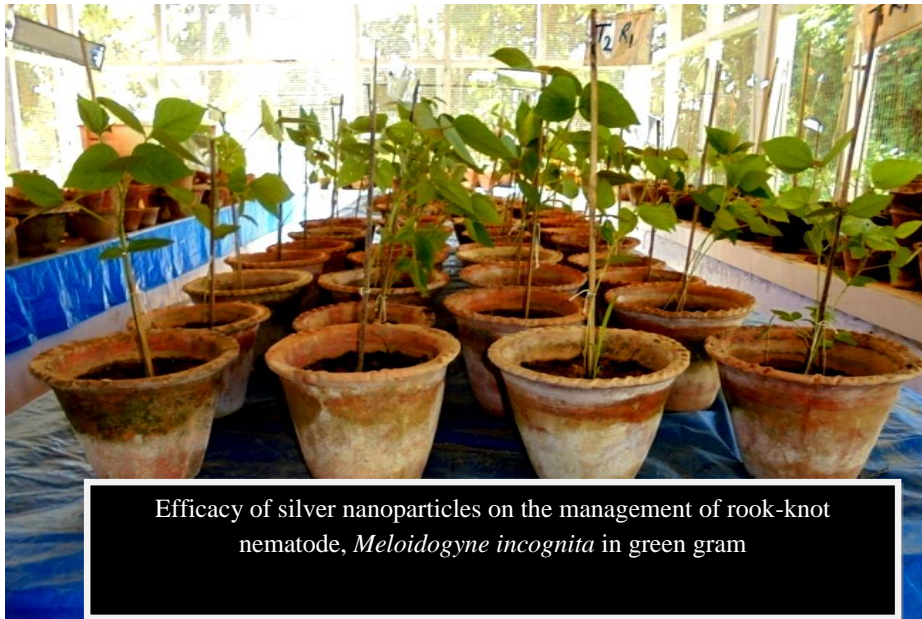
**Table 2. Effect of AgNPs on the development of *M. incognita* in green gram**

Treatments	No. of galls	No. of egg masses	Final nematode population/250cc soil	% decreased over control
T <sub>1</sub> : Control (UC)	0.00	0.00	0.00	-

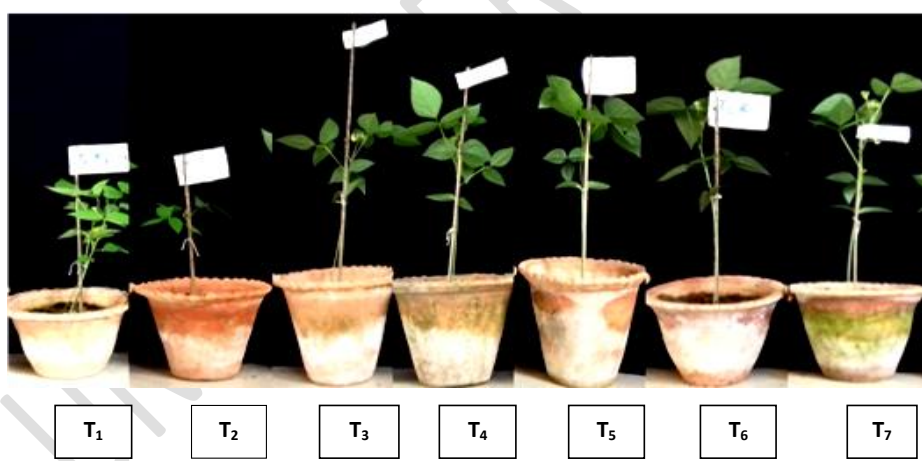
	(0.71) <sup>g</sup>	(0.71) <sup>g</sup>	(0.71) <sup>g</sup>	
T <sub>2</sub> : Control ( Nematode alone )	116.80 (10.74) <sup>a</sup>	46.60 (6.80) <sup>a</sup>	501.8 (22.36) <sup>a</sup>	-
T <sub>3</sub> :0.0015 ppm + <i>Meloidogyne incognita</i>	91.00 (9.48) <sup>b</sup>	35.60 (5.98) <sup>b</sup>	415.2 (20.30) <sup>b</sup>	17.25
T <sub>4</sub> : 0.003 ppm + <i>M. incognita</i>	46.40 (6.78) <sup>c</sup>	17.40 ( 4.02) <sup>c</sup>	319.0 (17.70) <sup>c</sup>	36.42
T <sub>5</sub> : 0.015 ppm + <i>M. incognita</i>	27.20 (5.20) <sup>d</sup>	13.20 (3.64) <sup>d</sup>	251.0 (15.80) <sup>d</sup>	49.98
T <sub>6</sub> : 0.03 ppm + <i>M. incognita</i>	15.60 (3.96) <sup>e</sup>	9.80 (3.14) <sup>e</sup>	228.8 (15.10) <sup>e</sup>	54.40
T <sub>7</sub> : 0.15 ppm + <i>M. incognita</i>	7.00 (2.68) <sup>f</sup>	5.80 (2.46) <sup>f</sup>	206.2 (14.38) <sup>f</sup>	58.90
S. Ed. (±)	0.34	0.16	0.25	-
CD <sub>0.05</sub>	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

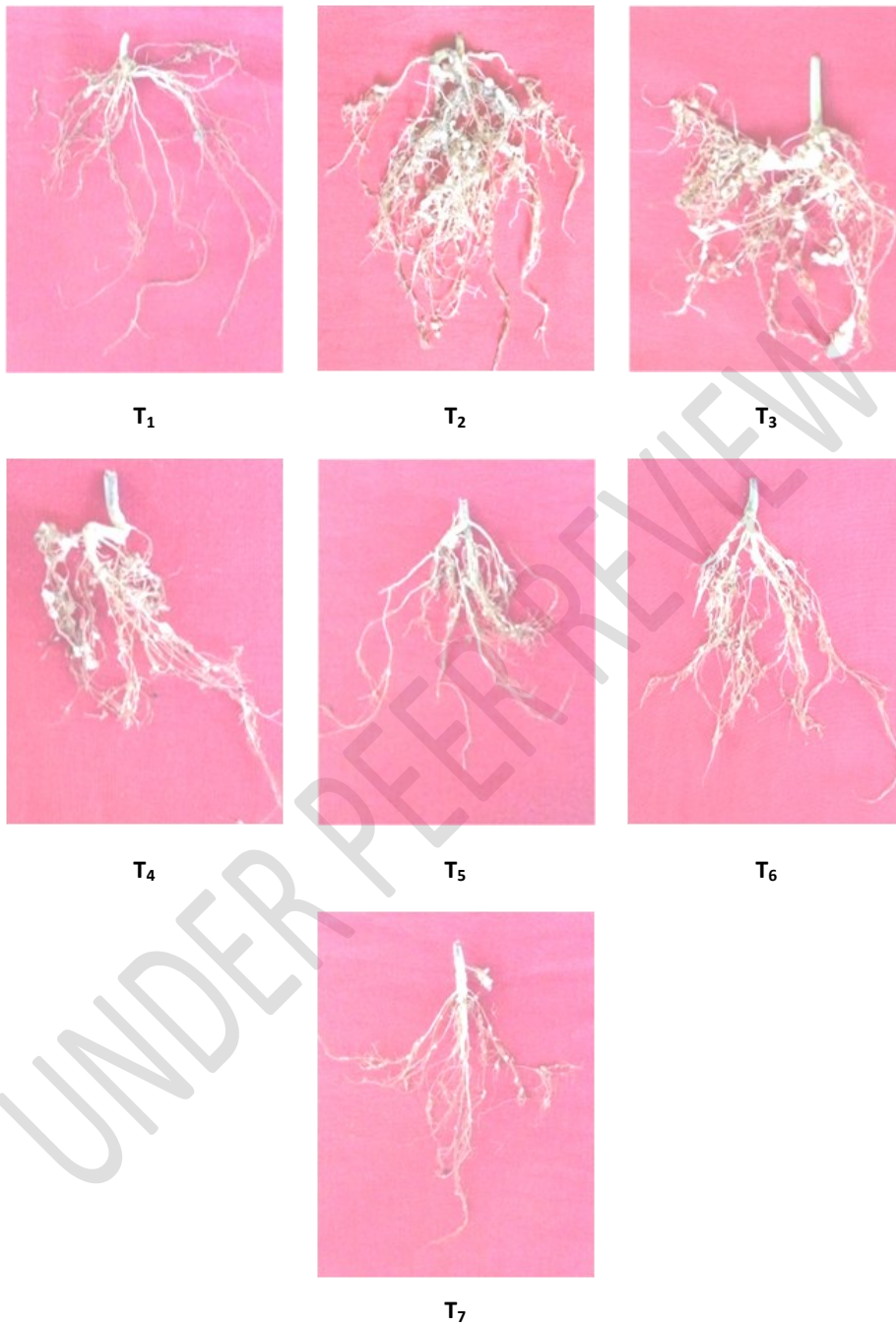
Mean followed by the same letter in the superscript(s) are not significantly different



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



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



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

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**Comment [H7]:** Please follow the authors guide for the proper referencing style