Original Research Article

Nano-mediated Management of Root-Knot Nematode (*Meloidogyne incognita*) in Okra: A Novel Approach for Sustainable Crop Protection

ABSTRACT

An experiment was conducted to study the effect of nanocomposite on plant growth parameters and multiplication of *Meloidogyne incognita* on okra. All the treatments improved the plant growth parameters in okra and reduced number of galls per root system, egg masses per root system, number of eggs per egg mass and final nematode population in soil except for the treatment with inoculated control (Nematode only). Among all the treatments, the treatment with AgNP @ 50 ppm + ZnONP @ 50 ppm was found to be the best treatment in increasing plant growth parameters while the treatment with carbofuran @ 1.5 g/pot and seeds treated with AgNP @ 50 ppm + ZnONP @ 50 ppm was effective in reducing root galls, egg masses, number of eggs per egg mass and final nematode population in soil. While analysing the biochemical changes, seeds treated with AgNP @ 50ppm + ZnONP @ 50 ppm had significantly increased nitrogen, phosphorus, potassium, total soluble sugar, and total free amino acids contents compared to all the treatments.

*Keywords: N*anocomposite *(Ag NP+* ZnONP*); Meloidogyne incognita, okra*

Introduction

Intherealmofscience,nanotechnology,oftenknownas “nanoparticlescience”, isanew and promising approach that deals with the usage and application of materials and devices whose smallest functional organisation is at least one dimension on the nanometer scale (one billionth of a meter (1-100 nm). Nanotechnology, can be used to manage soil borne pathogens including nematodes due to their ultra sub microscopic size, nanoparticles have gain the high

degree of reactivity and sensitivity and thus have potential to prove very useful in controlling root- knot nematode and other plant parasitic nematodes (Cromwell *et al.,* 2014). Nanomaterials may be used to deliver pesticides active ingredients, host defence agents, and other substances to the target pathogen in a way similar to how chemical pesticides act against pathogen. Broad spectrum, biocidal characteristics, antibacterial, antifungal, insecticidal and nematicidal effects are all attributes of silver and zinc oxide nanoparticles(Mukhtar et al., 2017). They are good plant growth stimulators and have potent inhibitory effect towards the plant pathogen(Singh et al., 2012). Nanoparticles have the potential to be used in preservation and exploitation of natural resources as well as in the crop production and livestock protection. Silver nanoparticles (AgNPs), gold nanoparticles (AuNPs), copper nanoparticles (CuNPs), zinc oxide nanoparticles (ZnO) and silicon carbide nanoparticles etc. have been reported to have nematicidal effect against root-knot nematode and considered as an effective means for controlling plant parasitic nematodes (Cromwell *et al.,* 2014; Baronia*et al*., 2020; Agwu&Ezigbo, 2005).

Among these nanoparticles, AgNPs is one of the most commonly used designed NPs in a variety of consumer products and it is projected to enter natural ecosystems, including soil through a variety of different pathways (Anjum *et al.,* 2013). It is reported that biosynthesized AgNPs have nematicidal activity and the effectiveness of Bio-AgNPs have shown decreased nematode activity, mortality, egg hatching, and larval migration (Ganaie et al., 2011).

Zinc oxide nanoparticles (ZnO-NPs) are among the NPs, that are used in several industries, medicine and agriculture because of its unique properties. As a result of direct interaction with ZnO-NPs, it may affect the organism, causing physical damage. ZnO-NPs used *in vivo* experiment confirmed that it was effective in reducing the nematode community and to promote banana health (Elansary*et al.,* 2021).

Composite nanoparticles are nanomaterials having composite structure made up of two or more components with unique physical and chemical properties at the nanoscale. Due to their relevance in science and technology, these advanced materials are used in crop protection, particularly in nematode management.

Nanoparticle's chemical and physical properties are very different from those of larger forms, thus understanding how they affect nematodes is crucial to properly assess the benefits of this technology in plant protection. As a result developing a reliable and

environmentally acceptable procedure for nanoparticles is a significant step in the field of nanotechnology application. However, it is only recently that microorganism have been looked into as a potential bio factory for synthesis of metallic nanoparticles such as cadmium sulphide, gold and silver. AgNPs are reported to be potentially hazardous 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 plant growth pattern (Shoot and root length, antioxidant and enzymes contents) found in corn, common beans (Salama, 2012). It was also observed that AgNPs showed the maximum level of root growth promotion (RGP) (Syu*et al.*, 2014).

Limited work so far has been done on the effect of nanocomposite against root-knot nematode, *Meloidogyne incognita* infecting okra in Assam except for the work done by Kumari*et al*., (2024) and Phukan, (2021) on the Efficacy of silver nanoparticles on the management and development of root-knot nematode, *Meloidogyne incognita* in green gram. There is lack of comprehensive information on nanotechnology application for controlling root-knot nematode. Therefore, based on the advantages of nanoparticles the present investigation is an attempt to study the Effect of Nanocomposite against root-knot nematode, *Meloidogyne incognita* infecting okra .

A pot experiment was conducted in the net-house of the Department of Nematology to study the quantitative changes on various factors and biochemical factors influenced by *M. incognita* on nanoparticles treated seeds. Seeds of okra (variety Parbhanikranti) were sown in pots containing 1 kg sterilized soil (2 parts soil, 1 partFYM and 1 part sand). After germination, thinning was done and one healthy seedling was kept in each pot. Seedlings were inoculated with nematode @ 1000 freshly hatched J_2 of *M. incognita* ineach pot.

Materials and Method

Research design

The design of experiment, no. of treatments and replication taken in the experiment were:

Design - CRD

No. of Treatments -6

Replications - 5

Pot Size - 1 kg capacity

Variety – Parbhanikranti

Treatments

T_1- AgNP @ 100 ppm

T_2- ZnONP @ 100 ppm

T_3- AgNP @ 50 ppm + ZnONP @ 50 ppm

T_4- Carbofuran @ 1.5g/ Pot

T_5- Inoculated control (Nematode only)

T_6-Control

There were 5 replications of each treatment. Till the time of harvest, regular irrigation was done. Carefully 60 days old plant were uprooted and roots were cleaned thoroughly under the tap water to get rid of any soil particles adhering to them. Observations on different plant growth parameters were recorded. The following parameters were recorded from the pot experiment: Shoot length, fresh weight of shoot, dry weight of shoot, fresh weight of root, dry weight of root, number of galls per root system, number of egg masses per root system, number of eggs per egg mass and final nematode population in soil. For measuring dry weight, the shoots and roots were put in an oven at a constant temperature of 60°C. Weighing was done regularly after every 24 hours until a constant weight was recorded. Final nematode population was recorded by drawing 250 cc of soil from a homogenous mixture of the entire soil in a pot and then processing the drawn 250 cc of soil

by Cobb's modified sieving technique (Christie and Perry, 1951). The different weather parameters were recorded during the period of experimentation.

Biochemical analysis

For biochemical analysis, oven dried roots were ground, powdered in an electric micro-grinder and used for estimation of various biochemical constituents like total nitrogen (N), phosphorus (P), potassium (K), total soluble sugar and total free amino acids.

The total nitrogen content was estimated by auto nitrogen analyzer, KJELTEC 1030.

Phosphorus content of root sample were estimated by Vanadomolybdophoric yellow colour method.

To 2 ml of stock solution in a 50 ml volumetric flask, 10 ml of vanadomolybdate reagent was added and the solution was diluted to 50 ml with distilled water and mixed well. After 10 minutes, the intensity of colour was measured in a spectrophotometer at 470 nm against a reagent blank. The concentration of phosphorus was calculated from the standard curve and expressed as percentage (%).

Estimation of potassium (K) was done by Flame photometer method.

One ml stock solution was taken in a 50 ml volumetric flask and the solution was then diluted to 50 ml by adding distilled water. Potassium content was determined by flame photometer. Concentration of potassium was calculated from the standard curve and expressed in percentage (%).

Total soluble sugar was estimated by using Anthrone method.

Moisture free powdered sample (200 mg) was extracted with 80% hot ethanol in a 50 ml centrifuge tube by stirring constantly with a glass rod. After centrifugation, the supernatant was collected in a 100ml volumetric flask. Supernatant was mixed together and the process was repeated twice and. The total volume was increased to 100 ml using distilled water.

To 1 ml of aliquot of the sugar extract 4 ml of freshly prepared anthrone (0.2%) was added slowly by the side of the test tube, placing the test tube in ice cold water and kept for 10 minutes. The test tubes were then placed in boiling water bath for half an hour and cooled immediately to room temperature. The intensity of colour was measured in a spectrophotometer at 630 nm against a reagent blank. The sugar content was estimated from a standard curve, prepared with known concentration of glucose and expressed as mg sugar per 100mg of moisture free dry root sample.

Total free amino acids were estimated by using ninhydrin reagent. Ninhydrin, a powerful oxidizing agent, decarboxylates the α-amino acids and yields an intensely coloured bluish purple product which is colorimetrically measured at 570nm.

To an accurately weighed powdered sample (0.5g) about 5 ml of 80% ethanol was added and ground it with a pestle and mortar. The homogenate was centrifuged at 10, 000 rpm for 10 minutes and saved the supernatant. The sample was re-extracted with 5 ml of 80% ethanol, centrifuged and pooled all the supernatants. The supernatant was evaporated to reduce to volume and this extracts were used for quantitative estimation of total free amino acids.

To 1 ml of aliquot of extract, 1.32 ml of ninhydrin solution (1%), 0.53 ml of citrate-buffer 0.5 M, pH 5.5 and 3.16 ml of glycerol were added; mixed the contents in the test tubes. Then, the test tubes were placed in boiling water bath for 30 minutes until bluish purple colour developed. After cooling, the intensity of colour was measured in a spectrophotometer at 570 nm against a reagent blank. The total freeamino acid in different test tubes were estimated from a standard curve prepared by using known concentration of glycine and expressed as mg/g dry root sample.

Results and discussions

The findings of the present investigation, as shown in the Table 1, revealed that the maximum plant growth parameters *viz.*, shoot length (61.40 cm), fresh weight of shoot (53.34 g), dry weight of shoot(8.44 g), fresh weight of root (8.63 g) and dry weight of root (3.89 g) were recorded in treatment with AgNP @ 50 ppm + ZnONP @ 50 ppm. While the minimum shoot length (41.00 cm), fresh weight of shoot (32.16 g), dry weight of shoot(4.10 g), fresh weight of root (4.26 g) and dry weight of root (1.34 g) were recorded in inoculated control

(Nematode only). According to Pankaj and Singh (2012) the use of silver nanoparticles resulted in increased shoot length. Also according to Hassan *et al*. (2016) application of a mixture of Oxamyl and nanoparticles under controlled greenhouse conditions resulted in an increase in shoot length upto 40 cm..El- Deen and El- Deeb (2018) reported that use of AgNPs improved plant growth in comparison to silver nitrate and control treatments. Kalaiselvi *et al.* (2019) they observed that plant treated with Et-AgNPs resulted in healthier plant growth parameters. Kumari *et al*., (2024) found increased plant growth parameters due to the application of silver nanoparticles

The minimum number of galls per root system, egg masses per root system, eggs per egg mass and final nematode population in soil was observed in the seeds treated with AgNP @ 50 ppm + ZnONP @ 50 ppm and treatment with carbofuran @ 1.5g/pot(Table- 2). All the treatments have shown reductions in number of galls, egg masses, eggs per egg mass and final nematode population. The seeds treated with AgNP @ 50 ppm + ZnONP @ 50 ppm reduced galls, egg masses and final nematode population by 72.34%, 48.29% and 61.44% and the treatment with carbofuran @ 1.5g/pot reduced galls, egg masses and final nematode population by 77.04%, 61.54% and 63.71% respectively over inoculated control (Nematode only). All the treatments was considerably different from the inoculated control (Nematode only). Similar findings were noticed by Pankaj and Singh (2012) reported that silver nanoparticles application resulted in a considerable decrease in tomato root galls. Cromwell *et al*. (2014) in Bermuda grass found that the percentage of healthy J2 decreased with increase in exposure time and concentration of AgNPs. Sharaf *et al.* (2016) showed that the treatments with silver nanoparticles against root-knot nematode, *Meloidogyne incognita* in tomato plants led to a reduction of root galls and eggmasses by 86% and 88% respectively. Taha(2016) reported that use of AgNPs suppressed nematode growth, galls, egg masses, final nematode population and egg hatchability in tomato plant. Kumari (2017) observed that AgNPs was effective in improving plant growth parameters and reducing galls, egg masses and final nematode population in soil.El- Deen and El- Deeb (2018) noticed lowest numbers of galls and egg-masses when tomato plants was treated with AgNP produced by ginger extract at 1 mM. Khalil *et al.* (2018) conducted an in vitro experiment on tomato galled roots infected by *Meloidogyne incognita* using SiO_2, ZnO, and Ag nanoparticles (NPs) where they found the egg hatching was significantly reduced and there impact increase with increasing the NPs concentration (250, 500, 1000 ppm***)*** and exposure period (24, 48, 72 hours after treatment). Kalaiselvi *et al.* (2019) observed that root dip treatment of plant with Et-

AgNPsresulted in decreased egg masses, number of eggs per egg mass, overall gall formation and J2 population in soil. Elansary*et al.* (2021) reported that applications of ZnO-NPs were more effective to control RKNs than ZnO-bulk as well oxamyl alone (chemical control). Kalaba *et al.* (2021) found that ZnO-NPs had a significant effect on *Meloidogyne incognita*, with death percentages of 88.2, 93.4 and 96.72% after 24, 48 and 72 h of exposure, respectively.

Biochemical changes

The results on biochemical changes (Table 3) showed that treatment with AgNP @ 50 ppm + ZnONP @ 50 ppm had significantly increased nitrogen (N), phosphorus (P) and potassium (K) contents in the roots of okra over the treatment with control and inoculated control (Nematode only). A significant increase in nitrogen, phosphorus and potassium contents was also observed in the treatment with ZnONP @100 ppm and AgNP @ 100 ppm. The percent increase in N, P and K contents were maximum in the treatment with AgNP @ 50 ppm + ZnONP @ 50 ppm (33.03%, 75.35% and 63.35%, respectively) and lowest N,P,K content was found in inoculated control (Nematode only), because nematode infection cause plants to drain out of these primary nutrient. Similar observations also reported by El- Sherif *et al.* (2007); Abdallah *et al.* (2010); Xu *et al.* (2010); Venkatesan *et al.* (2013) according to them plants infested with *M. incognita* significantly caused reduction in nitrogen, phosphorus and potassium contents. Tauseef *et al.* (2021) reported that 100 ppm dose of MgO nanoparticles, as root dip application enhanced plant growth, and increased the nitrogen contents of root and shoot.

Total soluble sugar content were also found to be increased significantly in the treatment with AgNP @ 50 ppm + ZnONP @ 50 ppm as compared to the treatment with control and inoculated control (Nematode only) or the treatment with ZnONP @ 100 ppm or AgNP @ 100 ppm or carbofuran @ 1.5 g/pot. The treatment with ZnONP @ 100 ppm also showed significant increase in total sugar over that of carbofuran @ 1.5 g/pot and inoculated control. But there was no significant difference in total sugar content in treatments with ZnONP @ 100 ppm and AgNP @ 100 ppm; AgNP @ 100 ppm and carbofuran @ 1.5 g/pot; carbofuran @ 1.5 g/pot and inoculated control. Maximum increase of total sugar (34.26%) was observed in the treatment with AgNP @ 50 ppm + ZnONP @ 50 ppm, followed by ZnONP @ 100 ppm (30.86%) and AgNP @ 100 ppm (29.60%). The increase in sugar

content following nematode infection confirms the findings of earlier researchers by Rao *et al.* (1988); Verma *et al.* (1996). Similar results of increase sugar content in plant were also reported by Gautam and Poddar (2014) and Singh *et al.* (2020).

Theresults also revealed that the treatment with AgNP @ 50 ppm + ZnONP @ 50 ppm had significantlyincreased total free amino acid contentin the roots of okra compared to the treatment with control and inoculated control (Nematode only) or the treatment with ZnONP @ 100 ppm or AgNP @ 100 ppm or carbofuran @ 1.5 g/pot. The total free amino acid content were also found to be significantly increased in the treatment with ZnONP @ 100 ppm over that of AgNP @ 100 ppm and carbofuran @ 1.5 g/pot. However, there was no significance difference was found between the treatments with AgNP @ 50 ppm + ZnONP @ 50 ppm and ZnONP @100 ppm; carbofuran @1.5g/pot and inoculated control (Nematode only). The percent increase in total free amino acid over inoculated control (Nematode only) were maximum in the treatment with AgNP @ 50 ppm + ZnONP @ 50 ppm (69.65%), followed by ZnONP @ 100 ppm (68.53%), AgNP @ 100 ppm (62.38%) and carbofuran @ 1.5g/pot (59.19%). Root- knot nematode secrete protease enzyme which results in break down of protein into amino acids (tryptophan) in the host tissues. Due to the break down of protein into amino acids there is more accumulation of free amino acids in the infection site of root- knot nematode. The presence of higher concentration of free amino acids in plants might be responsible for the resistance in plants against nematode infestation. These findings are in agreement with those ofMyuge (1956) in his work on amino acids in nematodes, he noted that the increase in amino acids at the feeding sites was caused by faster protein break down and delayed uptake of the same by the nematodes. Lewis and Mc clure(1975) compared the amount of free amino acids in cotton root segments in resistant and susceptible to *Meloidogyne incognita* and found that the total amount of free amino acid was more in the resistant cultivar. Masood and Hussain (1975) also reported higher concentrations of amino acids in resistant tomato cultivar as compared to that of susceptible one. Krishnaraj*et al.* (2012) reported that the increased concentration of secondary metabolites with reduced nematode and pest incidence observed in the nanoparticle treated plants might be due to stimulation of antioxidant system of plant upon treatment with nanoparticle there by improves the plants resistance by production of defense related compounds.

Higher content of N, P, K nutrients, total soluble sugar and total free amino acid observed in the present study might be due to the smaller sized of nanoparticles that are

absorbed easily and efficiently by the plants because of their higher surface area (Brakhage, 2013).

Conclusion

All the treatments recorded significantly higher plant growth compared to inoculated control (Nematode only).

The best result was obtained in the treatment with AgNP @ 50 ppm + ZnONP @ 50 ppm and carbofuran @ 1.5 g/pot in increasing plant growth parameters and reducing galls, egg masses, egg per egg mass and final nematode population in soil.

The plants with treatment AgNP @ 50 ppm + ZnONP @ 50 ppm had significantly increased the nitrogen (N), phosphorus (P), potassium (K) content in the root of okra over control and inoculated control (Nematode only) plants.

The total soluble sugar and total free amino acids contents were greatly increased in plants and the best result was recorded in treatments with AgNP @ 50 ppm + ZnONP @ 50 ppm followed by ZnONP @ 100 ppm, AgNP @ 100 ppm, carbofuran @ 1.5 g/pot over inoculated control (Nematode only) and control.

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Table 1. Effect of nanocomposite on plant growth parameters of okra infected by *Meloidogyne incognita* **Means of 5 replications**

Treatments	Shoot length (cm)	% increase over inoculated control	Fresh weight of shoot (g)	% increase over inoculated control	Dry weight of shoot (g)	% increase over inoculated control	Fresh weight of root (g)	% increase over inoculated control	Dry weight of root (g)	% increase over inoculated control

T_1 :AgNP @ 100 ppm	55.00[c]	25.45	45.33[c]	29.05	7.25[c]	43.49	7.39[c]	42.21	2.91[c]	53.85
T_2 :ZnONP @ 100 ppm	58.20[b]	29.55	49.52[b]	35.04	7.79[b]	47.41	7.89[b]	45.98	3.45[b]	61.00
T_3 : AgNP @ 50 ppm + ZnONP @ 50 ppm	61.40[a]	33.22	53.34[a]	39.69	8.44[a]	51.45	8.63[a]	50.58	3.89[a]	65.41
T_4 : Carbofuran @ 1.5 g/pot	52.00[d]	21.15	42.44[d]	24.21	6.69[d]	38.81	6.62[d]	35.57	2.31[d]	41.82
T_5 : Inoculated control (Nematode only)	41.00[f]	-	32.16[f]	-	4.10[f]	-	4.26[f]	-	1.34[f]	-
T_6 : Control	49.80[e]	17.67	39.77[e]	19.13	6.13[e]	33.20	6.00[e]	28.98	2.06[e]	34.69
CD (0.05)	2.178	-	2.19	-	0.52	-	0.50	-	0.24	-
SEd±	1.06	-	1.06	-	0.25	-	0.24	-	0.12	-

Mean values shown in superscript(s) are significantly different

Table 2. Effect of nanocomposite on multiplication of *Meloidogyne incognita* in okra

Mean of 5 replications

Treatments	**No. of galls per root system**	**% decrease over inoculated control**	**No. of egg masses per root system**	**% decrease over inoculated control**	**No. of eggs per egg mass**	**Final nematode population in soil per 250 cc soil**	**% decrease over inoculated control**

T_1 :AgNP @ 100 ppm	55.80 *(7.49)[b]	61.41	37.20 *(6.13)[b]	20.51	97.80 *(9.91)[b]	225.40 *(15.03)[b]	56.67
T_2 :ZnONP @ 100 ppm	47.20 *(6.91)[c]	67.36	30.00 *(5.52)[c]	35.90	89.40 *(9.48)[c]	211.80 *(14.57)[c]	59.28
T_3 : AgNP @ 50 ppm + ZnONP @ 50 ppm	40.00 *(6.36)[d]	72.34	24.20 *(4.97)[d]	48.29	82.00 *(9.08)[d]	200.60 *(14.18)[d]	61.44
T_4 : Carbofuran @ 1.5 g/pot	33.20 *(5.80)[e]	77.04	18.00 *(4.30)[e]	61.54	76.60 *(8.78)[e]	188.80 *(13.76)[e]	63.71
T_5 : Inoculated control (Nematode only)	144.60 *(12.02)[a]	-	46.80 *(6.86)[a]	-	183.00 *(13.55)[a]	520.20 *(22.82)[a]	-
T_6 : Control	0 *(0.71)[f]	-	0 *(0.71)[f]	-	0 *(0.71)[f]	0 *(0.71)[f]	-
CD (0.05)	6.22		5.57		4.45	5.62	
SEd±	3.02		2.70		2.16	2.72	

*Values within parenthesis are square root $\left(\sqrt{x+0.5}\right)$transformed data

Mean values shown in superscript(s) are significantly different

Table 3. Effect of nanocomposite on biochemical changes on the roots of okra infected by *Meloidogyne incognita*

Mean of 5 replications

Treatments	**N (%)**	**% increase over**	**P (%)**	**% increase over**	**K (%)**	**% increase over**	**Total soluble sugar (mg/100m**	**% increase over**	**Total free amino acid**	**% increase over**

		N		N		N	g dry weight)	N	(mg/g dry weight)	N
T_1 :AgNP @ 100 ppm	1.62[c]	23.08	0.35[c]	59.65	0.58[c]	51.81	2.97[bc]	29.60	0.61[b]	62.38
T_2 :ZnONP @ 100 ppm	1.69[b]	26.58	0.46[b]	69.50	0.61[b]	53.72	3.02[b]	30.86	0.73[a]	68.53
T_3 : AgNP @ 50 ppm + ZnONP @ 50 ppm	1.86[a]	33.03	0.57[a]	75.35	0.76[a]	63.35	3.18[a]	34.26	0.76[a]	69.65
T_4 : Carbofuran @ 1.5 g/pot	1.55[d]	20.01	0.25[d]	43.09	0.50[d]	44	2.95[cd]	29.12	0.57[c]	59.19
T_5 : Inoculated control (Nematode only)	1.24[f]	-	0.14[e]	-	0.28[f]	-	2.89[d]	-	0.54[c]	-
T_6 : Control	1.47[e]	15.67	0.21[d]	33.96	0.38[e]	26.89	2.09[e]	-	0.23[d]	-
CD (0.05)	0.03		0.04		0.002		0.05		0.03	
SEd±	0.02		0.02		0.02		0.03		0.02	

Means followed by the same letter shown in superscripts(s) are not significantly different