Effect of nanocomposite on penetration and development of *Meloidogyne incognita* on okra.

ABSTRACT

Studies on the effect of nanocomposite on penetration and development of *Meloidogyne incognita* on okra revealed that the penetration of nematode started within 24 hours of inoculation in all the treatments and inoculated control (Nematode only). The seeds treated with AgNP @ 50 ppm + ZnONP @ 50 ppm and treatment with carbofuran @ 1.5 g/pot were found to be most effective in reducing J_2 penetration, number of galls per root system, eggmasses per root system, final nematode population in soil and delayed the development of *Meloidogyne incognita* as compared to the treatments with ZnONP @ 100 ppm, AgNP @ 100 ppm and inoculated control (Nematode only).

Keywords: Nanocomposite (Ag NP+ ZnONP); penetration ; development; *Meloidogyne incognita*; okra

INTRODUCTION

The root knot nematode, *Meloidogyne incognita* is one of the most prevalent species of nematode associated with vegetable crops in India(Swarup, 1962; Seshadri, 1970). In addition, Root knot nematodes interact with particular fungi and bacteria to produce disease complexes, causing additional yield reduction and break down resistance against the pathogens (Begum *et. al*., 2012), which lowers the level of tolerance in plants to environment stress. Okra yield losses up to 27 per cent have reportedly been attributed by the root knot nematode (Sikora and Fernandez, 2005).Many people avoid growing

okra because of risk of association with root knot nematode (Marin *et. al*.; 2017). Although using chemical is wide spread and it is common approach to manage nematodes, the use of these nematicides may result in a poor cost benefit ratio, generate environment pollution, pose a health risk and also conventional nematicides might not be available. Therefore, it has been prioritised to look better alternatives and methods that are both sustainable and economically feasible for the management of root knot nematodes. Over the last few decades, the use of nanoparticles (NP's) in agriculture has grown immense popularity, this is because nanoparticles are widely used in the environment due to their unique, optical, electronic and magnetic properties. Accordingly, using nanoparticles (NP's) to control plant pathogen is a novel approach that may be beneficial in the future as nanotechnology application developed. Ghazala(2004) carried out an experiment and revealed that cadmium inhibited root penetration by the second stage juveniles (J2) of *Meloidogyne incognita* which subsequently affected the development of root galls in tomato. Hawk and Travis (2020) reported that flow of fluopyram significantly reduced *Meloidogyne incognita* penetration, gall development and reproduction compared to the non treated control in both cotton and soybean. Kumari *et. al.* (2024) recorded maximum reduction in galls, eggmasses and final nematode population in the treatment with 0.15ppm AgNPs. No work so far has been done on the effect of nanocomposite on penetration and development of Meloidogyne incognita in Okra in Assam. Therefore, the study was done on effect of nanocomposite on penetration and development of Meloidogyne incognita in Okra.

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MATERIALS AND METHODS

A pot experiment was carried out in the net house of the Department of Nematology. Soil was collected from Instructional Cum Research (ICR) farm and sterilized. Pure culture of *M. incognita* was maintained and inoculum was collected. Overnight treatment of okra seeds with silver nanoparticles, zinc oxide nanoparticles and nanocomposite of silver and zinc oxide nanoparticles were done and carbofuran was mixed in the pot one day prior to the sowing of okra seeds in 1kg capacity plastic pots. After germination of okra seeds, thinning was done and kept one healthy seedling in each pot. Ten days old seedlings were inoculated with freshly hatched J_2 of *M. incognita*. Seedlings inoculated with nematode alone was taken as control. The treatments taken in the experiments were:

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)

Each treatment was replicated 5 times for each observation. Fifty five number of pots for each treatment were maintained for recording the observations on 11 different days (1, 3, 7, 9, 12, 15, 18, 21, 24, 27 and 30th day) after nematode inoculation.

Observation on nematode penetration were recorded on 1st, 3rd, and 7th day of nematode inoculation. From 9th day onwards observations was taken at 3 days interval upto 30 days for penetration and development studies. Five plants from each treatment were uprooted carefully, roots were then washed and stained with lactophenol-acid fuchsin and examined under stereoscopic binocular microscope for juvenile (J_2) penetration and developmental stages of *M. incognita*. The different

developmental stages *viz.* 3rd, 4th and adult females were recorded. The number of galls per root system, number of egg masses per root system and final nematode population in soil were recorded on 30th day of nematode inoculation.

RESULTS AND DISCUSSIONS

The results obtained in the study on the effect of nanocomposite on penetration of *M. incognita* on okra indicated that the penetration of J_2 started within 24 hours of inoculation in all the treatments that is, seeds treated with nanoparticles, soil treated with carbofuran and inoculated control (Nematode only) (Table- 1). Further, the penetration of juveniles increased with increase in days of observation with maximum number of J_2 penetration on 7th day after inoculation in all the treatments including inoculated control (Nematode only). The maximum number of J_2 penetration was recorded in inoculated control (Nematode only). While the minimum penetration was recorded in the treatment with carbofuran @ 1.5g/pot and seeds treated with AgNP @ 50 ppm + ZnONP @ 50 ppm in all observation days *viz.,* 1st, 3rd and 7th days after inoculation. However, no significant difference was observed among the treatments with AgNP @ 100 ppm and ZnONP @ 100 ppm and also between the treatments with AgNP @ 50 ppm + ZnONP @ 50 ppm and carbofuran @1.5 g/pot but these treatments significantly reduced the penetration of J_2 into the host roots as compared to the treatments with AgNP @ 100 ppm, ZnONP @ 100 ppm and inoculated control (Nematode only). The treatments with AgNP @ 100 ppm, ZnONP @ 100 ppm and AgNP @ 50 ppm + ZnONP @ 50 ppm were at par in reduction of J_2 penetration. Similar results on reduction in nematode penetration also observed by Sharma and Trivedi (1994) who reported reduced larval penetration in okra and Windham and

Williams (1994) reported that penetration of second stage juveniles (J_2) of *Meloidogyne incognita* at 3 DAI was similar for the four corn genotypes, and root-knot nematode numbers in four genotypes of corn *viz.* Tebeau, old Raccon, Mp307, and Pioneer 3110 was peaked at 12, 12, 15, and 27 DAI, respectively. Ghazala (2004) revealed that cadmium metal was highly injurious to tomato plants at different concentrations tested at (7.5, 15.0, 30.0 and 60.0 ppm), which inhibited the root penetration of second stage juveniles (J_2) of *Meloidogyne incognita* and subsequently affected the development of root galls in tomato. Hawk and Travis (2020) reported that fluopyram significantly reduced nematode penetration, gall development, and reproduction compared to the non-treated control in both cotton and soybean. Anwar *et al.* (2021), who reported that the J_2 penetration on the root of pointed gourd started after 24 hour of inoculation and 58.5% penetration was recorded in roots while 90.12% penetrated J_2 were moulted into different stage of juveniles however, moulting was started from third day and development of young females from 18th day after inoculation. The juvenile's stages (J_3 and J_4) become sedentary (Table 2). Maturation of females was started from 20th to 22nd days. The present study have shown that seeds treated with nanoparticles pre-disposes the host root to increase plant resistance to nematode infection and may not be favourable for penetration and multiplication of root-knot nematode larvae of AgNP @ 50 ppm + ZnONP @ 50 ppm and carbofuran @ 1.5 g/pot, 15-21 days in the treatment with AgNP @ 100 ppm and ZnONP @ 100 ppm and 15-18 days in treatment with inoculated control (Nematode only). Adult females were observed from 24-30 days in the treatment of ZnONP @ 100 ppm, AgNP @ 50 ppm + ZnONP @ 50 ppm and carbofuran @ 1.5 g/pot, 21-30 days in the treatment with AgNP @ 100 ppm and inoculated control (Nematode only). However, the maximum number of J_2, J_3, J_4 and adult females were recorded on 7th, 15th, 18th and 30th day of inoculation, respectively (Table 4).

The results (Table 3and 4) on the effect of nanocomposite on the development of *M. incognita* on okra showed that treatments with AgNP @ 50 ppm + ZnONP @ 50 ppm and carbofuran @ 1.5 g/pot delayed further development of J2 as compared to the treatments with AgNP @ 100 ppm, ZnONP @ 100 ppm and inoculated control (Nematode only). The maximum J_3, J_4 and adult females were recorded in inoculated control (Nematode only) which differed significantly from all the other treatments. The minimum number of J3, J4 and adult females were recorded in the treatment with carbofuran @ 1.5 g/pot which is followed by AgNP @ 50 ppm + ZnONP @ 50 ppm, ZnONP @ 100 ppm and AgNP @ 100 ppm. Further, the treatment with carbofuran @ 1.5 g/pot showed minimum number of galls per root system, eggmasses per root system, and final nematode population in soil, while the maximum was recorded in inoculated control (Nematode only). The treatments with AgNP @ 100 ppm, ZnONP @ 100 ppm and AgNP @ 50 ppm + ZnONP @ 50 ppm were found to be effective in reducing the number of galls, eggmasses, and final nematode population in soil over that of inoculated control (Nematode only). The treatment with carbofuran @ 1.5 g/pot and seeds treated with AgNP @ 50 ppm + ZnONP @ 50 ppm was found to be most effective in reducing nematode penetration and development in the roots of okra. These findings are in agreement with those of Sharma and Trivedi (1994) also observed similar type of results working on tomato, which resulted in reduced larval penetration, development, number of galls and eggmasses per plant. Esmenjaud *et al.* (1999) who reported that the resistance phenomenon does not act on the very early nematode penetration but acts later by preventing feeding-site induction and development into the third-stage.

Walters *et al.* (2006) observed the Post-penetration development of *M. incognita*, which was delayed because of abnormal development of the feeding site for optimum nematode development in *Cucumis sativus* and *C. metuliferus* roots. Proite

et al. (2008) experimented on two wild species of Arachis (*A. duranensis* and *A. stenosperma*) and cultivated peanut (*A. hypogaea cv.* IAC-Tatu-ST) and found that penetration and development of root- knot nematode in the resistant species was reduced in comparision with that occurring in susceptible plants. Hamidi and Hajihassani (2020) also found that the development of the nematodes as evident from counting young and egg-laying females in roots were significantly decreased or inhibited in the resistant cultivars compared to the susceptible cultivars. Heflish *et al.* (2021) examined the nematicidal activity of biosynthesized Ag NPs conc. 25, 50, and 100 mg/mL and evaluated in vitro against root-knot nematode (*Meloidogyne incognita*), egg hatching (6 days later) and movement after 24 and 48 h. The Ag NPs (100 mg/ml) application after 48 hours was the most successful treatment that resulting in a 53.3% of nematode mortality. Overall, nematode activity, mortality, egg hatching, and larval migration were decreased due to the effectiveness of bio- Ag NPs. Li *et al.* (2021) studied the comparison between susceptible cucumber inbred line Q24 and the resistant CM, that after the infection of *M. incognita*, the 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.

Conclusion:

The developmental study also indicated that the treatments with AgNP @ 50 ppm + ZnONP @ 50 ppm and carbofuran @ 1.5 g/pot delayed further development of J2 of *M. incognita* as compared to the treatments with ZnONP @ 100 ppm, AgNP @ 100 ppm and inoculated control (Nematode only).

All the treatments *viz.,* AgNP @ 100 ppm, ZnONP @ 100 ppm, AgNP @ 50 ppm + ZnONP @ 50 ppm and carbofuran @ 1.5 g/pot were found to be effective in reducing number of galls, eggmasses and final nematode population in soil.

The best outcome was attained in the treatment with carbofuran @ 1.5 g/pot followed by seed treated with AgNP @ 50 ppm + ZnONP @ 50 ppm in reducing nematode penetration and development of *M. incognita,* number of galls, eggmasses, and final nematode population in soil.

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Table 1. Effect of nanocomposite on penetration of *Meloidogyne incognita* in the roots of okra

Mean of 5 replications

Treatments	Number of juveniles		
	1st day	**3rd day**	**7th day**
T$_1$: AgNP @ 100 ppm	1.40	8.80	14.20
	*(1.37)[b]	*(2.95)[b]	*(3.77)[b]
T$_2$: ZnONP @ 100 ppm	1.00	7.40	11.60
	*(1.23)[b]	*(2.71)[bc]	*(3.40)[c]
T$_3$: AgNP @ 50 ppm + ZnONP @ 50 ppm	0.80	6.60	10.80
	*(1.12)[bc]	*(2.56)[c]	*(3.28)[c]
T$_4$: Carbofuran @ 1.5 g/pot	0.40	5.00	8.80
	*(0.91)[c]	*(2.23)[d]	*(2.96)[d]
T$_5$: Inoculated control (Nematode only)	2.80	18.60	33.00
	*(1.81)[a]	*(4.31)[a]	*(5.74)[a]
CD (0.05)	0.72	1.78	1.61
SEd±	0.35	0.85	0.77

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

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

Table2. Effect of nanocomposite on penetration and development of *Meloidogyne incognita* on okra

Treatments	Observation days			
	J_2	J_3	J_4	Adult female
T_1 : AgNP @ 100 ppm	1-9	9-15	15-21	21-30
T_2 : ZnONP @ 100 ppm	1-9	9-15	15-21	24-30
T_3 : AgNP @ 50 ppm + ZnONP @ 50 ppm	1-12	12-18	18-21	24-30
T_4 : Carbofuran @ 1.5 g/pot	1-12	12-18	18-21	24-30
T_5 : Inoculated control (Nematode only)	1-9	7-15	15-18	21-30

Table 3. Effect of nanocomposite on the development of *Meloidogyne incognita* in okra

Treatments	Developmental stages		
	J_3	J_4	Adult female
T_1 : AgNP @ 100 ppm	22.60 *(4.75)[b]	15.80 *(3.97)[b]	19.80 *(4.45)[b]
T_2 : ZnONP @ 100 ppm	17.00 *(4.12)[c]	12.20 *(3.49)[c]	15.40 *(3.92)[c]
T_3 : AgNP @ 50 ppm + ZnONP @ 50 ppm	13.80 *(3.71)[d]	10.20 *(3.19)[d]	12.40 *(3.52)[d]
T_4 : Carbofuran @ 1.5 g/pot	10.80 *(3.28)[e]	7.40 *(2.71)[e]	9.20 *(3.03)[e]
T_5 : Inoculated control (Nematode only)	32.80 *(5.73)[a]	25.60 *(5.06)[a]	29.00 *(5.39)[a]
CD (0.05)	1.51	1.41	1.32
SEd±	0.74	0.68	0.63

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

  Mean values shown in superscript(s) are significantly different

Table 4. Effect of nanocomposite on the development of *Meloidogyne incognita* in okra

<div align="right">Mean of 5 replications</div>

Treatments	No. of galls per root system	% decrease over inoculated control	No. of egg masses per root system	% decrease over inoculated control	Final nematode population (250 cc soil)	% decrease over inoculated control
T_1 : AgNP @ 100 ppm	47.20 *$(6.87)^b$	60.65	29.00 *$(5.38)^b$	33.79	221.40 *$(14.88)^b$	56.40
T_2 : ZnONP @ 100 ppm	39.40 *$(6.27)^c$	67.17	25.00 *$(5.00)^c$	42.92	205.80 *$(14.35)^c$	59.47
T_3 : AgNP @ 50 ppm + ZnONP @ 50 ppm	30.80 *$(5.55)^d$	74.33	20.00 *$(4.47)^d$	54.34	190.60 *$(13.81)^d$	62.41
T_4 : Carbofuran @ 1.5 g/pot	25.20 *$(5.02)^e$	79.00	15.40 *$(3.91)^e$	64.84	181.00 *$(13.45)^e$	64.36
T_5 : Inoculated control (Nematode only)	120.00 *$(10.95)^a$	-	43.80 *$(6.61)^a$	-	507.80 *$(22.53)^a$	-
CD (0.05)	4.83		3.78		6.75	
SEd±	2.31		1.81		3.24	

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

Mean values shown in superscript(s) are significantly different