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Effect of a Newly Developed Bio-Nematicide in Controlling Root-Knot (*Meloidogyne incognita*) of Okra (*Abelmoschus esculentus*)

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Abstract: The development of new potential pesticides is essential to manage the parasitic nematodes worldwide which hinders plant growth and yield. Three preparations of a newly developed Bio-nematicide and a chemical nematicide Curaterr in eight treatments, including control, were tested against root-knot (*Meloidogyne incognita*) of okra. All preparations of the Bio-nematicide and Curaterr were used as side dressing. Preparations of Bio-nematicide N_0D_2, N_1D_2, and Curaterr gave better results in plant growth characters, consequently with less galling and nematode development compared with untreated control. Protective (seed coating @ 10g/kg seed) and curative (soil application @ 10g/plant and 20g/plant) significantly decrease the egg masses number and gall number/plant on okra roots. Bio-nematicide (N_0D_2) showed the best performance with the highest length of shoot and root, fresh weight of shoot and root, number and weight of fruits per plant correspondingly with decreased number of galls, **egg masses,** adult females and juveniles of the nematode. Negative correlation between gall numbers with shoot and root length, weight of shoot and root, and weight and numbers of fruits plant-1 indicate the positive response of the treatments on okra against the root-knot nematode. These suggest that the bionematicide formulation has nematocidal properties and would have the potential to control root-knot nematodes. Therefore, farmers could adopt it as an alternative to carbofuran or other synthetic nematicide chemicals in controlling root-knot nematode on farmlands.

Keywords: Bio-nematicide; Root-knot nematode; Okra; Treatment; Biological control.

INTRODUCTION

Okra *(Hibiscus esculentus* L.) is a widespread vegetable crop grown in Bangladesh. It can be grown in homesteads and kitchen gardens. It is one of the economically important crops for small farmers in Bangladesh. Green fruits are consumed as vegetables, and they are highly nutritious and rich in vitamins (A, B, and C) and minerals (Schippers, 2002). Okra is also considered a good source of calcium and potassium (Duvauchelle, 2011).

In spite of increasing demand for okra consumption in Bangladesh its production is very limited. The causes for less cultivation and yield is due to not practicing standard procedures, procuring uncertified seeds, lack of pesticides knowledge which leads to susceptible to many diseases including root knot caused by *Meloidogyne incognita*.

The Appropriate and specific control measures have yet to be adopt in Bangladesh to save okra and other common vegetable crops from the root knot disease. The Use of chemicals (Enokpa et al, 1996 and Jada et al., 2011), cultural practices (Bari et al., 1999) and biological (Faruk *et al.*, 2001 and Faruk *et al.*, 1999) means may control the disease. The continuous and indiscriminate use of chemicals for controlling disease of crop plants leads to environmental pollutions which also instigate public health hazards. The entailed and the advancement of technologies in bio-controls that are sustainable and ecologically friendly, which could

minimize the use of synthetic fertilizers (Santoyo et al., 2012; Zhang et al., 2021). Henceforth, beneficial microbiomes application for the sustainable practices of agriculture has appeared as cutting-edge technology for betterment of soil fertility, productivity and plant growth (Adesemoye et al., 2009; Bertola et al., 2019; Ullah et al., 2019a; Murgese et al., 2020; Fasusi et al. 2021). The other factor of using chemical fertilizers are, it is all expensive and most of them are imported which burdens the farmer which directly affects the Bangladesh economy. Researchers demonstrated that microbes will be an alternate source for these problems, biological controls through beneficial microorganisms have gained significant importance. Currently, several biological control agents (BCAs) have been screened, among them *Bacillus, Pantoea, Streptomyces, Trichoderma, Clonostachys, Pseudomonas, Burkholderia*, and certain yeasts (Lahlali et al.2022) showing promising results. Many investigators attempt to control root-knot of various crops with neem products with full or partial success (Firoza and Maqbool, 1996; Wani, 1992). The neem trees are commonly widely available in Bangladesh and can be utilized as an organic derivative, which will be cost effective against root-knot diseases. The neem derived organic pesticide compounds decreases the root galling and can influence the management of *M. incognita* infestation in plants.

In place of pesticides, biological control of plant diseases provides long-lasting, economical, and environmentally friendly solutions (Papavizas and Lumsden, 1980; Tariq et al., 2020). The environment surrounding a crop plant is manipulated to encourage the organism that contributes to plant health and vigor in biological control, which is essentially different from chemical control of plant infections.In biological control, live microorganisms such as bacteria, viruses or fungi are employed as antagonists, parasites, or predators (Kwok *et al*., 1987). *Trichoderma* spp. is also known as antagonists to plant pathogenic nematodes and control egg masses of nematode very effectively (Sarker *et al.*, 2020). In addition, it also has been found to stimulate the growth of the plants (Inber *et al.*, 1994).

Recently, carbofuran gains more attention among Bangladeshi farmers which is also formulated locally. It controls the nematodes efficiently, but it is costing more to the farmers. The other disadvantages include the dosage application. The farmers ignorantly apply more or less dose which leads to deposition and environmental pollution. Thus, the use of chemicals is being discouraged all over the world. The okra is an important crop in the country and little attention has been given to control the root-knot disease by biological means leads to maintain natural harmony. Therefore, this experiment done with nematicidal formulation with *Trichoderma harzianum* (TH), as Trichoderma can directly parasitize nematode, and neem oil and leaf mash (Zhang et al., 2017).

MATERIALS AND METHODS

Preparation of Soil and Potting

The Seed Pathology Centre (SPC), Department of Plant Pathology, Bangladesh Agricultural University (BAU), Mymensingh, Bangladesh, served as the site for the studies. Initially, well-decomposed cow dung, sand, and sandy loam soil were combined in a 3:1:1 ratio. We gathered soil, sand, and cow manure from the Bangladesh Agricultural University campus, the Brahmaputra River, and the Bangladesh Agricultural University dairy farm, respectively. After that, 30 milliliters of formalin were dissolved in 1000 milliliters of water per cubic foot of soil to sterilize the mixture. A polythene covering was placed over the formalin-treated soil and left undisturbed for 72 hours. To eliminate the formalin vapor, the polythene layer was taken off after 72 hours and the sterile soil was left to air-dry for 48 hours. Forty 30-cm-diameter earthen pots were selected, and each one was filled with 6 kg of dried and sterilized dirt and a tiny fragment of shattered earthen pot on the bottom hole.

Collection of Seeds

Okra seeds that are robust, disease-free, and healthy of BARI Okra-1 variety were collected from Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur. Bangladesh

Collection of *Trichoderma harzianum*

The mother Culture of *Trichoderma harzianum* was collected from IPM Laboratory, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh, Bangladesh.

Collection of nematicide Curaterr (Carbofuran)

Granular nematicide Curaterr was collected from the local market of Mymensingh town, Bangladesh in 1 kg packets.

Surface sterilization, sowing of okra seeds & Aftercare of seedlings

The okra seeds were surface sterilized with 10% chlorox solution for 30 seconds. Subsequently, the seeds were rinsed and washed with sterilized distilled water for three times. After surface sterilization the seeds were potted in different pots. Each pot received three apparently healthy and uniformly treated seeds after coating with nematicidal formulation @ 20g/kg of okra seed. The seeds in the pots are well maintained for proper growth with suitable moisture by regular watering of the pots. After seven days' germination was observed from the seeds, and we sustained only one seed per pot, and others were removed. Continuous monitoring, weeding, irrigation, mulching etc. were done as and when necessary. We also conserved pots from the incidence of disease or insect attacks. No fungicidal or insecticidal spray was applied.

Collection of Egg Masses and Preparation of Inoculum

The mature egg masses of the root-knot nematode (*Meloidogyne incognita*) were extracted from brinjal

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(*Solanum melongena*) root systems that were severely galled. We injected the fresh, healthy, disease-free brinjal plants with a single mass of *M. incognita* eggs. The Department of Plant Pathology at the Bangladesh Agricultural University in Mymensingh, Bangladesh, provided glasshouse conditions for the brinjal plants to develop in huge earthen pots with a diameter of 25 cm. Using fine forceps, the reddish-brown mature egg masses were extracted from infected roots once the eggs had matured in the brinjal plant. Following a one-minute sterilization with 10% Clorox, the egg masses were gathered and placed in a damp petri dish surface before being cleaned with sterile water.

Inoculation of okra plants

After 15 days of planting, each okra seedling pot emerged as plants and was inoculated with the eight egg masses collected from infected brinjal plants. On either side of the plant, four egg masses were inoculated over the exposed roots of the seedlings under the soil from the base of the plant.

Preparation and Application of Bio-nematicide

Bio-nematicide was prepared by mixing mustard oil cake, *Trichoderma harzianum* culture, poultry litter, neem leaf mash, neem oil, decaffeinated tea waste, and bran of black gram. Bio-nematicide applied as side dressing at 10g plant^{-1} [Dose 1 (D_1)] and 20g plant^{-1} [Dose 2 (D_2)] around the root region of seedlings after 15 days of inoculation with the egg masses.

Table 1: Different ingredients in three preparations of Bio-nematicide

Sl. No.	Ingredients	Bio-nematicides Composition		
		N_0	N_1	N_2
1	Mustard oil cake	200g	200g	150g
2	*Trichoderma harzianum* culture	------	150g	100g
3	Poultry litter	150g	150g	100g
4	Neem leaf mash	100g	100g	75g
5	Neem oil	100g	100g	75g
6	Decaffeinated tea waste	150g	150g	100g
7	Bran of blackgame	300g	150g	400g
Total amount		1000g	1000g	1000g

Figure 1. Three Preparation of Bionematicide

Granular Nematicide Application:

Curaterr (Carbofuran) was applied as side dressing at 500 mg plant^{-1} around the root region of seedlings after 15 days of inoculation with egg masses for our control experiments.

Experimental Design

The experiment was set up in the insect-controlled poly house at Seed Pathology Centre, Bangladesh Agricultural University, Mymensingh, Bangladesh. All the okra plant pots were organized on the floor under the shade. We made eight treatments including control and we also replicated each treatment five times, with total 40 pots. It was one factor

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factorial experiment conducted in Randomized Complete Block Design (RCBD).

Eight treatments used in our experiments are given below:

Variant Parameters

After 60 days of egg mass inoculation, the plants at mature stage were carefully uprooted from the pots and the following parameters in relation to plants and pathogens were studied:

Shoot and root length and weight measurement

Initially, the soil of the pots was watered to uproot the plants. Consequently, the whole plant, along with soil attached to its roots was uprooted from the pot and dipped in a bucket water to remove the soils from the roots. The roots were again cleaned under gentle running tap water to remove the complete debris. The root portion was separated from the shoot portion with a sharp knife; length of shoot was measured from the base of the stem up to the topmost leaf. Similarly, length of root was measured from the starting point of the root to the largest available lateral root apex. The shoot and root portions were blotted with fine tissue paper and fresh weights were measured by electrical balance before it gets desiccated.

Enumeration of Galls of Root

The roots of individual plants were cut into small pieces of 1 cm in size and randomly one gram of fresh root was taken from the bulk quantity to count the number of galls formed. The average number of galls g^{-1} of root was counted from five replicated plants. After then, the roots were preserved in 5% formalin solution for further process.

Enumeration of egg masses g^{-1} of root

The roots of each individual plant were cut into small pieces and randomly one gram of fresh root was taken from the bulk to count the number of egg masses formed. The average number of egg masses g^{-1} of the root was counted from five replicated plants. The roots were preserved in 5% formalin solution for prospective analysis.

T_0 = Control	N_0= Bio-nematicide prepared without
T_1= N_0D_1	*Trichoderma harzianum*
T_2 = N_0D_2	N_1= Bio-nematicide prepared with 150g
T_3 = N_1D_1	*Trichoderma harzianum*
T_4 = N_1D_2	N_2= Bio-nematicide prepared with 100g
T_5 = N_2D_1	*Trichoderma harzianum*
T_6 = N_2D_2	D_1= 10g Bio-nematicide/Plant
T_7= Curaterr	D_2= 20g Bio-nematicide/Plant

Statistical Analysis

All the data were analyzed statistically to find out the level of significance using the statistical computer package program MSTATC. Duncan's New Multiple Range Test (DMRT) was applied to evaluate the mean differences for their significant level. Linear correlation co-efficient was also performed.

RESULTS

The effects of the eight treatments used in the experiment were found to be significant ($P \geq 0.05$) in respect of plant growth characteristics and galling incidence. The effects were also found to be significant ($P \geq 0.05$) on the population development of egg masses, adult females, J_2, J_3, J_4 juveniles of *Meloidogyne incognita* in okra.

Effects of different treatments on the plant growth, yield and galling incidence of okra

Data on plant growth, yield and galling incidence of okra inoculated with *Meloidogyne incognita* are illustrated as in Table 2

Shoot length

The length of shoot was significantly influenced by the treatments. Mean length of shoot ranged from 42.34 cm to 72.80 cm. The highest shoot length was recorded with treatment T_2 having 72.80 cm followed by treatment T_4 and T_7 having 65.80 cm and 61.40 cm, respectively. The lowest length of shoot was found with T_0 having 42.34 cm preceded by T_3, T_1, T_5 and T_6 having 47.86 cm, 48.20 cm, 51.68 cm and 53.64, respectively. But statistically no significant difference was found among the treatments T_1, T_3, and T_5 (Table 1; Plate2).

Root length

Like that of shoot length, plant under treatment T_2 gave the maximum root length 33.92 cm followed by T_4, T_7 and T_1 having 25.06 cm, 24.32 cm and 23.24 cm respectively. Significantly, the lowest and identical length of root was found with the treatment T_0 and T_5 having 17.16 cm and 17.16 cm, respectively. Comparatively, higher root length was found with the treatments T_1, T_4 and T_7. There was no significant difference among the treatments T_1, T_4, and T_7 (Table 2; Plate 3a, Plate 3b and Plate 3c).

Shoot mass

Fresh weights of shoot ranged from 37.40 g to 87.76 g. Significantly the highest shoot weight was found in plants treated with T_2 having 87.76 g followed by plants treated with T_7 and T_4 having 71.24 g and 67.22 g respectively. Significantly the lowest shoot weight was recorded with T_0having 37.40 g. But there was no significant difference among the treatments T_4, T_6 and T_1 as well as the treatments T_3 and T_5 having respectively67.22g, 62.64g and 60.16g as well as 51.12g and50.60g (Table 2).

Fresh root mass

The maximum fresh weight of root was noted with treatment T_2 having 17.64 g followed by T_7, T_4 and T_6 having 14.54 g, 14.12 g and 13.18 g, respectively. Significantly lower and statistically similar fresh weights of root were observed with the treatments T_0 and T_3 having 8.96 g and 9.74 g, respectively. Among the treatments no significant difference was found in T_4 and T_6 as well as T_4 and T_7, but there was significant difference between T_6 and T_7. Again,

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there was no significant difference between the treatments T_1 and T_5 (Table 2).

Fruits per plant enumeration

Maximum number of fruits 4.80 plant^{-1} was recorded with treatment T_2 followed by T_4, T_7 and T_1 having 4.20, 3.40 and 3.20 fruits plant^{-1}. The lowest number of fruits plant^{-1} was found with the treatment T_0 having 1.60 fruits plant^{-1}. But there was no significant difference among the treatments T_2 and T_4 having 21.14 cm and 24.28 cm, respectively. Again, there was no significant difference among the treatments T_0, T_3, T_5 and T_6 having 1.60, 2.40, 2.60 and 2.60, respectively. Similarly, there was no significant difference between the treatments T_7 and T_1 (Table 2).

Fruits per plant mass

The highest fruit weight, 59.18 g plant^{-1} was obtained from the plants with treatment T_2 followed by T_4 and T_7 having 48.12 g and 41.80 g fruit plant^{-1}, respectively. Minimum fruit weight 17.76 g was recorded in the treatments T_0. Statistically identical response was found among the treatments T_1, T_5 and T_3 having 37.66 g, 36.22 g and 32.68 g, respectively. No significant difference was found between the treatments T_4 and T_7 having 48.12g and 41.40 g, respectively. Again, there was significant difference among the treatments T_1, T_3, T_5 and T_6 (Table 2).

Galls per gram of root estimation

The control treatment T_0 was found to have significantly the highest number 4.40 of galls g^{-1} of root followed by T_5 and T_6 having 2.80 and 2.40 galls g^{-1} of root, respectively. Minimum number of galls g^{-1} of root was recorded in the treatment T_2 having 1.40 preceded by T_7, T_4, T_3 and T_1 is having 1.60, 1.80, 2.00 and 2.00 galls g^{-1} of root, respectively. But there was no significant difference among the treatments T_1, T_3 T_4 and T_7 same was true for the treatments T_5 and T_6 (Table 2).

Table 2: Effects of different treatments on the plant growth, yield and galling of okra inoculated with *Meloidogyne incognita* after 60 days of inoculation

Treatments	Length of shoot (cm)	Length of root (cm)	Weight of shoot (g)	Weight of root (g)	Number of fruits plant^{-1}	Weight of fruits plant^{-1}	Number of galls g^{-1} of root
T_0 (control)	42.34 f	17.16 e	37.40 e	8.96 e	1.60 d	17.76 e	4.40 a
T_1 (N_0D_1)	48.20 e	23.24 bc	60.16 c	11.78 d	3.20 bc	37.66 cd	2.00 cd
T_2 (N_0D_2)	72.80 a	33.92 a	87.76 a	17.64 a	4.80 a	59.18 a	1.40 d
T_3 (N_1D_1)	47.86 e	21.22 cd	51.12 d	9.74 e	2.40 cd	32.68 cd	2.00 cd
T_4 (N_1D_2)	65.80 b	25.06 b	67.22 bc	14.12 bc	4.20 ab	48.12 b	1.80 cd
T_5 (N_2D_1)	51.68 de	17.24 e	50.60 d	11.60 d	2.60 cd	36.22 cd	2.80 b
T_6 (N_2D_2)	53.64 d	20.40 d	62.64 c	13.18 c	2.60 cd	32.02 d	2.40 bc
T_7 (curaterr)	61.40 c	24.32 b	71.24 b	14.54 b	3.40bc	41.80bc	1.60 d
Sx$^-$	1.392	0.715	2.522	0.442	0.331	3.017	0.247

Each value is an average of five replications; Values in the same column having common letter(s) do not differ significantly at 5% level of significance by DMRT.

Effects of different treatments on the development of egg masses and the growth of *Meloidogyne incognita* in the inoculated okra plants

Effects of eight different treatments on the development of egg masses, adult females, J_2, J_3 and J_4 juveniles of *Meloidogyne incongnita* in the inoculated okra plants are presented in Table 3.

Estimation of egg masses per gram of root

Significant variations were observed among the treatments in respect of number of egg masses g^{-1} of root. The highest number of egg masses g^{-1} of root was recorded with treatment T_0 having 6.20 followed by treatment T_1, T_5 and T_6 has 4.40, 4.00 and 3.60, respectively. The lowest number of egg masses g^{-1} of root was recorded in the treatment T_2 having 1.80 followed by T_4 having 2.40 egg masses g^{-1} of root. However, there was no significant difference among the treatments T_1, T_5 and T_6. Similarly, there were no significant differences among the treatments T_3, T_5 and T_6. Again, there were no significant differences among the treatments T_3, T_6 and T_7. (Table3).

Validation of adult females per 10 galls

Maximum number 2.80, of adult females of *Meloidogyne incognita* was found with the treatment T_0 followed by T_1, T_5, T_6, T_3 and T_7 having 2.00, 2.00, 1.80, 1.40 and 1.40, respectively. Minimum number of adult female was 0.60 / 10

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galls. But no significant differences were found among the treatments T_1, T_5, T_6, T_3 and T_7 (Table 3).

Assessment of J2 juvenile per 10 galls

Like that of adult females, plants under treatment T_0 gave the highest number 1.00 of J_2 juvenile/10 galls followed by T_1, T_3, T_5, T_4, T_6, T_7 and T_2 having 0.80, 0.80, 0.80, 0.60, 0.60, 0.60 and 0.20 J_2 juvenile/10 galls, respectively. Minimum number 0.20 of J_2 juvenile/10 galls was found with the treatment T_2. There was no significant difference among T_1, T_2, T_3, T_4, T_5, T_6 and T_7. But significant difference was found between the treatments T_0 and T_2 (Table 3).

J3 juvenile per 10galls

Treatment T_0 and T_5 appeared to have significant and statistically identical number of J_3 juvenile/10 gall having 2.00 and 1.80 J_3, respectively. The lowest number 0.60 of J_3 juvenile/10 galls was found with the treatments T_2 preceded by T_4, T_7, T_3, T_6 and T_1 having 0.80, 1.00, 1.40, 1.60 and 1.60, respectively. There was no significant difference among the treatments T_0, T_1, T_3, T_5 and T_6. Again, no significant differences were found between the treatments T_2, T_4 and T_7. But significant difference was found between the treatments T_2 and T_0 as well as T_2 and T_5 (Table 3).

Assessment J4 juvenile per 10galls

LikethatofJ_3juvenile'splantsundertreatmentT_0gavemaximumnumber2.00of J_4 juvenile/10galls followed by having statistically similar number1.40 of J_4 juveniles in the treatments T_1, T_5 and T_6 as well as T_3 having 1.20 J_4 juveniles. Minimum number 0.60 of J_4 juvenile/10 galls was found with the treatment T_2 preceded by T_4 and T_7 having 0.80 and 1.00, respectively. But there was no significant difference among the treatments T_2, T_4 and T_7 in respect of J_4 population. Again, significant difference was recorded between the treatments T_0 and T_2 (Table3).

Table 3: Effects of different treatments on the development of egg masses, adult females and juveniles of *Meloidogyne incognita* in the infected okra plants after 60 days of inoculation

Treatments	Number of egg masses g^{-1} of root	Number of adult females per 10 galls	Number of J_2 juveniles per 10 galls	Number of J_3 juveniles per 10 galls	Number of J_4 juveniles per 10 galls
T_0 (Control)	6.20 a	2.80 a	1.00 a	2.00 a	2.00 a
T_1 (N_0D_1)	4.40 b	2.00 b	0.80 ab	1.60 ab	1.40 b
T_2 (N_0D_2)	1.80 f	0.60 d	0.20 b	0.60 d	0.60 c
T_3 (N_1D_1)	3.40 cd	1.40 bc	0.80 ab	1.40 abc	1.20 b
T_4 (N_1D_2)	2.40 ef	1.00 cd	0.60 ab	0.80 cd	0.80 bc
T_5 (N_2D_1)	4.00 bc	2.00 b	0.80 ab	1.80 a	1.40 b
T_6 (N_2D_2)	3.60 bcd	1.80 b	0.60 ab	1.60 ab	1.40 b
T_7 (Curaterr)	3.00 de	1.40 bc	0.60 ab	1.00 bcd	1.00 bc
S x	0.268	0.227	0.201	0.225	0.190

DISCUSSIONS

The present study reveals that the was carried out with eight different treatments covering with a newly developed Bio-nematicide with its three preparations in two doses (N_0D_1, N_0D_2, N_1D_1, N_1D_2, N_2D_1, N_2D_2), a chemical nematicide Curaterr and a control to see their effect on the plant growth characters, yield components, galling incidence and development of egg masses, adult females, J_2, J_3 and J_4 juveniles in okra var. BARI okra-1 inoculated with *Meloidogyne incognita*. Bio-nematicide and Curaterr were used as side dressing.

The experiment showed that the synthesis of Bio-nematicide (N_0D_2) treatment, followed by Bio-nematicide (N_1D_2) and chemical nematicide Curaterr, produced the highest length of shoot and root, fresh weight of shoot and root, and number and weight of fruits per plant. However, the number and weight of fruits per plant, as well as the length and fresh weight of the shoots and roots, were all significantly reduced in the control treatment, which was only based on *Meloidogynae incognita*. The control treatment had the highest galling incidence and, consequently, the lowest yield performance.

Plant metabolism was less disturbed by the nematode, as evident by the higher growth and yield components correspondingly with lower galling incidence, egg masses, and nematode development with the application of the new Bio-nematicide (N_0D_2).

The newly developed Bio-nematicide and Curaterr preparations N_0D_2 and N_1D_2 demonstrated superior responses, exhibiting increased shoot and root growth and weights that corresponded to increased fruit weight and quantity. Both preparations of the bio-nematicide N_0D_2 and N_1D_2 treated plants showed a markedly reduced galling incidence, suggesting that they had the same galling-suppressing effect as the chemical nematicide Curaterr. Furthermore, fewer adult females were discovered using these bio-nematicide preparations, and their reactions were relatively comparable to those of the chemical Curaterr. In comparison to the preparations N_0D_2, N_1D_2, and chemical

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Curaterr, the treatments containing the bio-nematicides N_2D_1, N_2D_2, N_0D_1, and N_1D_1 exhibited worse performances in plant development features and yield appropriately with higher egg masses, adult females, J_4, J_3, and J_2 juveniles. According to Sharma et al. (2007) and Eifediyi et al. (2013), okra plants' growth metrics improved when treated with neem cake either by alone or in conjunction with carbofuran. According to Sharma et al. (2000), soaking okra seeds with neem compounds neemark and nimbecidine effectively inhibits the root knot nematode (M. incognita). Soaking seeds in neemark and nimbecidine resulted in a significant decrease in the amount of galls. According to Singh et al. (2003), Nimin, a neem product, enhanced the development characteristics of okra plants and drastically decreased the root knot index of the nematode population. According to Agbenin et al. (2004), neem seed powder dramatically decreased the disease severity of root knot in both greenhouse and field settings. According to Sivakumar and Ramakrishan (2005), neem oil produced robust and healthy seedlings and considerably decreased the root knot index.

As neem oil and neem leaf mash were components of the newly developed Bio-nematicide, the positive result obtained in the present study with different preparations of Bio-nematicide in respect of increased plant growth and yield with reduced galling incidence, egg masses and nematode juveniles in okra are likely to be antibiotic actions of these neem products as stated by the above authors.

A good number of researchers (Manuzca, 2001; Khan *et al.* 2001, Rakibuzzaman et al. 2021) reported that when plants inoculated with fungal agent *Trichoderma harzinum* had better development of leaves and roots than plants inoculated with nematodes alone. The suppressing effects on galling and nematode development as observed in the present study with the preparations of N_1D_1, N_1D_2, N_2D_1 and N_2D_1 of the Bio-nematicide correspondingly with better plant growth and fruit yield might be due to the nematicidal properties of the released non-volatile antibiotics and improved proteolytic activity of *Trichoderma harzianum*as already stated by the above authors. Sharon *et al.* (2001) suggested that improved proteolytic activity of the antagonist might be important for the biological control of the nematode. Increased plant growth characters, reduced galling incidence and nematode population with bio fungicides like *Trichoderma harzianum* and *T. viride* in different crops infected with root knot nematode has also been reported by the (Devi *et al,* 2002, Gopal-Pandey *et al.* 2003, Al-Ani, L. K. T. 2018). In the present experiment *Trichoderma harzianum*was added to the preparation of Bio-nematicide with N_1 (150 g) and N_2 (100 g) as components. Its addition to N_1 and N_2 preparation as bio-agent could not promisingly like N_0 where no *Trichoderma harzianum* was added. It might be due to toxic substances of mustard oil cake and poultry litter which hampered the individual action of *Trichoderma harzianum* on nematode activity under this synergistic condition as observed with less performances in plant growth and yield as well as reducing galls, egg masses and nematode population. Chaitali *et. al.* (2003) similarly observed fewer effective reactions of *T. viride* with groundnut cake in controlling the disease complex of root knot nematode *(Meloidogynae incognita)* and root knot fungus (*Rhizoctonia bataticola)* in okra cv. Pusa Sawani. Nafady et. al. (2022) also found *Trichoderma harzianum* can limit the growth and reproduction of the nematode and lessen the parasitism in tomato plant roots.

However, Jain (1990) reported that okra and tomato plants treated with nematicide Carbofuran had significantly lower root knot index than control. Enokpa *et al.* (1996) stated that Curbofuran increased the vegetative growth, plant dry weight and lowered the galling incidence. Sharma *et al.* (2001) reported the efficacy of the treatment with Carbofuran for the management of the root knot nematode *Meloidogyne incognita.* Curbofuran gave the best result in plant growth characters and suppressing nematode reproduction. Similar reports on the effectiveness of Carbofuran against root knot nematode have also been reported by Singh (2006), Hasseeb and Sukla (2004) and Fatema (2003) are agree with the present findings. Treatments with N_0D_2 and N_1D_2 preparations of bio-nematicide appeared with the better performances in respect of plant growth and yield along with reduced number of galls, egg masses and nematode populations. As both N_0 and N_1 preparations contain higher concentrations of mustard oil cake, poultry litter, neem leaf mash, neem oil and decaffeinated tea waste compared to N_2 preparation. The presence of higher quantity of all these bio-agents in N_0 and N_1 preparations made vulnerable situation for the nematodes by releasing antibiotic substances from neem as well as toxic substances like organic acids and phenolic compounds from mustard oil cake and poultry litter in the pot soil as evident with higher plant growth and yield. Working with saw dust, poultry manure and sesame oil cake as soil amendment against root knot nematode (*Meloidogyne incognita*) of tomato Radwan *et al.* (2004) similarly observed improved plant growth correspondingly with reduced galls and J_2 population. Faruk *et al.* (2001) also observed the efficacy of pre-plant treatment with poultry refuse and neem leaf powder for the management of root knot nematode *(Meloidogyne* spp.) of tomato under pot-house conditions and in field conditions. In experiments, poultry manure and neem leaf powder gave considerable reduction of root knot disease. The treatments also improved plant growth (weight and length of shoot and weight of root) in the field, significantly increased yield of tomato. Fathi *et al.* (2004) observed that control of tomato root-knot nematode (*Meloidogyne incognita*) using tea (*Camellia sinensis*) dust residues at different rates was quite effective. His result showed that the treatment with decaffeinated tea waste 25 g/kg soil effectively reduced gall index and increased the growth rates of tomato. Same was observed by Roy (1983). All these reports are agreed with the present findings.

The results of the study clearly showed that side dressing okra plants with the preparations N_0D_2 of Bio-nematicide, followed by N_1D_2 preparation and chemical nematicide Curaterr, resulted in higher plant growth characters in terms of length of shoot and root, fresh weight of shoot and root, and number and weight of fruits per plant correspondingly with reduced galling incidence and egg masses. The investigation suggested that using the recently created Bio-

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nematicide as a side dressing is a very efficient way to control *Meloidogynae incognita*. Therefore, the application of this recently produced Bio-nematicide for environmentally friendly treatment of this nemic disease, avoiding chemical nematicides as Curaterr, may be investigated for control of the root-knot disease of okra caused by *Meloidogynae incognita*. However, before making any recommendations to farmers, a field trial is necessary.

CONCLUSION

Root-knot disease controlled by means of chemical nematicide is not only costly but also detrimental to the environment as well as to human health. Our experiment showed that okra nematode can be effectively controlled by using environmentally safe bio-nematicide. But, more in depth research is necessary before making any recommendations. Taking into consideration the importance of okra as a vegetable crop and its greater yield loss because of root-knot disease, more importance has to be given to controlling the disease by biological means without disturbing nature.

Conflicts of Interests

The signing authors of this research work declare that they have no potential conflict of personal or economic interest with other people or organizations.

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