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Endophytic Fungi as Potential Bio-Control Agents against Root Knot Nematode, *Meloidogyne incognita* in Banana

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Authors' contributions

This work was carried out in collaboration among all authors. Authors PV, BB, NG and KD designed the study. Author KG performed the experiment, statistical analysis and wrote the first draft of the manuscript. Authors PV, KD, MR and DB supervised the study and corrected the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

A Survey was conducted in 12 districts of Assam to collect 92 healthy banana root samples. A total of 55 fungal isolates were successfully isolated from commercial banana cultivars. The culture filtrates were extracted from 55 endophytic fungal isolates and screened against root knot nematode, *Meloidogyne incognita in vitro* and pot culture studies. Among them, five fungal isolates *viz*., EF4, BF7, BF27, BF28 and BF35 showed 100% inhibition of egg hatching and 96.33 to 81.33% juvenile mortality of *M. incognita* with an exposure period of 72h when compared to other isolates and control. On paddy seed treatment with endophytic fungi of five promising isolates, two isolates *viz*., BF7 and BF28 significantly enhanced germination percentage (82.67%, 73.33%) and vigour index (62.91, 47.24%), respectively. The selected five endophytic fungal isolates were

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evaluated for their efficacy against *M. incognita* in banana under pot culture conditions. The study revealed that culture filtrates of BF7 and BF28 significantly reduced the soil and root nematode population, number of adult females, egg masses and root gall index of *M.incognita* compared to untreated control. The isolates BF7 and BF28 also significantly increased the growth parameters *viz*., pseudostem height, root length and pseudostem girth. These promising endophytic fungal isolates *viz*., BF7 and BF28 were identified as non-pathogenic *Fusarium oxysporum* strains (Accession no. MN567668) and (Accession no. MN567710), respectively by PCR -18S rRNA of ITS region of gene sequence and phylogenetic tree construction.

Keywords: Antinemic properties; Endophytic fungi; Fusarium oxysporum; growth promotion activity; Meloidogyne incognita.

1. INTRODUCTION

Root knot nematode, *Meloidogyne* spp. is the most destructive plant parasitic nematode act as sedentary endoparasite of banana, *Musa* spp. The second stage infective juveniles (J2) are parasite on young feeder root/ meristematic tissues of more than 3000 plant species [1]. Root knot nematode has the ability to establish the permanent feeding sites and root galls [2] by hypertrophy and hyperplasia, thereby caused yield loss of 15 per cent in banana production [3]. Chemical nematicides are presently used for management of nematodes, which have harmful effect on the environment including on beneficial soil microbiota. Due to the toxicity and residues of chemical nematicides, the effective, safe and non-chemical alternative method is need for suppression nematode population. Beneficial microorganisms like bacteria and fungi found to be effective in plant parasitic nematodes suppression by competition for food and space, production of toxins and secondary metabolites.

Endophyte includes bacteria or fungi have colonized plant tissues and live in plant cells in a symbiotic relationship. Among other endophytes, fungal endophytes such as *Beauveria bassiana*, *Metarhizium anisopliae*, *Clonostachys rosea*, *Isaria farinosa* and *Acremonium* sp., are the most common, varied and well-studied group for their role against biotic stress and stimulation of defense mechanism against pathogens [4-7]. The ability of a large variety of endophytic strains *Fusarium oxysporum* to be utilised as a biocontrol has been reported in many independent studies implying that it is a generic feature for *F. oxysporum* (Fo). This idea is supported by a study in which over 200 different non-pathogenic Fo strains isolated from a tomato field were able to confer biocontrol potential in tomato by tripartite interaction [8]. Antagonism of *F. oxysporum* towards *Helicotylenchus multicinctus*, *Meloidogyne incognita*, *Meloidogyne graminicola*, *Pratylenchus goodeyi* and *Radopholus similis* in banana, melons or tomato has been reported from different studies [9-12]. Other examples include *Acremonium coenophialum, Neotyphodium coenophialum* against *Pratylenchus scribneri*, *Meloidogyne marylandi* and *Helicotylenchus pseudorobustus* in tall fescue by Van Dessel *et al*., [13] and *Chaetomium globosum* towards *M. incognita* in cotton [14-17]. Therefore, biocontrol potential of endophytic fungi associated with banana has to be explored for the management of root knot nematode.

2. MATERIALS AND METHODS

A field survey was conducted to collect the healthy banana root samples for the isolation of endophytes from 12 districts of Assam *viz*., Jorhat, Golaghat, Nagaon, Marigaon, Goalpara, Dibrugarh, Tinsukia, Lakhimpur, Dhemaji, Sivsagar, Kamrup and Barpeta. A total of 92 root samples were collected from different banana cultivars *viz*., Malbhog, Seni Champa and Jahaji. The young feeder roots were collected at a depth of 20 to 30 cm and collected samples were packed in properly labeled zip lock cover then brought to the laboratory for fungal isolation. *In vitro* and pot culture studies was conducted at Department of Nematology, TNAU, Coimbatore.

2.1 Endophytes Isolation

The collected root samples were washed with tap water and cut into small pieces using a sterile blade. Root samples were surface sterilized with 5% NaOCl for 20 min, then washed twice with sterile distilled water to remove excess NaOCl. Subsequently washed with 70% ethanol for 30 sec. and several times with sterile distilled water,1 mL of sample was drawn and plated in Potato Dextrose Agar (PDA) medium as a sterile check. The root samples were homogenized using pestle and mortar by sterile peptone salt

(20 g of dextrose, 4 g (from 200 g infused potato) in 1 litre of water) 10 mL was used for maceration of a sample, allow it to withstand for 20 min and 1 mL of supernatant was taken from each sample for serial dilution. Three dilutions were carried out serially (10^1 to 10^3) for each sample and spread on the PDA.Two replications were maintained for each dilution. The PDA plates were incubated at 27 ± 2°C for week for the fungalmat growth.

2.2 *In vitro* Efficacy of Fungal Endophytes against *M. incognita*

Endophytic fungal isolates were grown on Potato Dextrose (PD) broth and incubated for seven days @ 27 ± 2°C. The fungal culture was centrifuged at 10000 rpm for 15 min (Centrifuge 5430R- Eppendorf) and the supernate filtered on a 0.2 µm Millipore filter device to obtain fungal cell free culture (FCFC). A total of 55 fungal isolates were screened against *M. incognita* under the laboratory conditions. For *in vitro* study, eggs and J2 of *M. incognita* was placed in a cavity block (100 nos.each/ cavity) with 2 mL of fungal cell free filtrate and incubated at 27 ± 2°C for studying egg hatching and juvenile mortality, respectively. The PD broth and distilled water were used as control. Observations were recorded on number of egg hatched and immobilized juveniles after 24, 48 and 72h of incubation. Three replications were maintained for each isolate and the experiment was arranged in a completely randomized design.

2.3 Testing of growth promotion

Among the 55 fungal isolates, five promising fungal endophytes were used for testing of growth promotion activities. Paddy cv.Co.52 seeds (25 nos.) were soaked in a small Petri dish 5cm containing 3mL fungal culture and assessed by a modified roll towel method [18]. The germination percentage, shoot length and root length was recorded at 25 days after germination. The Vigour index (VI) was calculated using the following formula [19]. VI = Germination percentage X Seedling length (shoot length + root length).

2.4 Evaluation of Fungal Endophytes Activity against *M. incognita*

The pot mixtures containing red soil, sand and farm yard manure (FYM) @ 2:1:1 ratio, respectively were prepared and sterilized in an autoclave and filled in the earthen pot. The banana cv. Ney Poovan suckers were trimmed and dipped with fungal culture @ 10mL/ sucker for 15- 20 minutes, then planted in 5 kg earthen pots at one sucker per pot. Freshly hatched 5000 J2 of *M. incognita* was inoculated per pot at 15 days after planting. Three replications were maintained for each isolate and the experiment was arranged in a completely randomized design. The observations on growth parameters and nematode multiplication factors were recorded 90 days after nematode inoculation.The collected soil and root samples were processed by Cobb's decanting and sieving method [20] and Modified Baermann funnel technique [21], respectively. The representative 5 g root samples of each pot was washed free of soil and stained with 0.1% of acid fuchsin - lactophenol to examine the number of females and egg masses. The root knot index was graded using 1 to 5 scale rating [22] *viz*., 1: no galls; 2: 1-25% galls; 3: 25-50% galls 4: 50-75% galls; 5; > 75% galls/ root system.

2.5 Molecular Identification of Promising Endophytes

The promising, two fungal endophytes which gave the highest inhibition of egg hatching and caused juvenile mortality of root knot nematode based on *in vitro* and pot culture studies were identified by PCR- 18S rRNA of ITS region sequence. The fungal genomic DNA was extracted from the culture of the two fungal endophytes using the commercially available DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the standard procedures. Amplification of the internal transcribed spacer (ITS) region was performed with primers ITS1F 5′-CTTGGTCATTTAGAGGAAGTAA-3′ and ITS4R 5′-TCCTCCGCTTATTGATATGC-3′ by [23]. PCR amplification of the target sequence was carried out with cycle of initial denaturation at 95°C for 2 min, 35 cycles of denaturation at 95°C for 1 min, annealing at 55°C, extension at 72°C for 1 min and a final extension of 72°C for 5 min. The PCR products were run on 0.8% agarose gel to check the amplified products. The PCR products were sent for sequencing at AgriGenome Labs Pvt Ltd. Kochi, Kerala. Chromatograms were manually edited and a consensus sequence was generated and subjected to Basic Local Alignment Search Tool (BLAST). Nucleotide sequences obtained were identified by comparison against the GenBank. Phylogenetic trees were constructed by the neighbor-joining method. Relevant sequences were collected and data plotted with MEGA 7

software. Selected isolates were identified at genus and species level from the Dendogram drawn by [24].

2.6 Statistical Analysis

All the experimental data were analyzed independently. The treatment means were compared by Duncan's Multiple Range-Test (DMRT) [25]. The package used for analysis was IRRISTAT version 92-1 developed by the International Rice Research Institute Biometrics unit, Philippines.

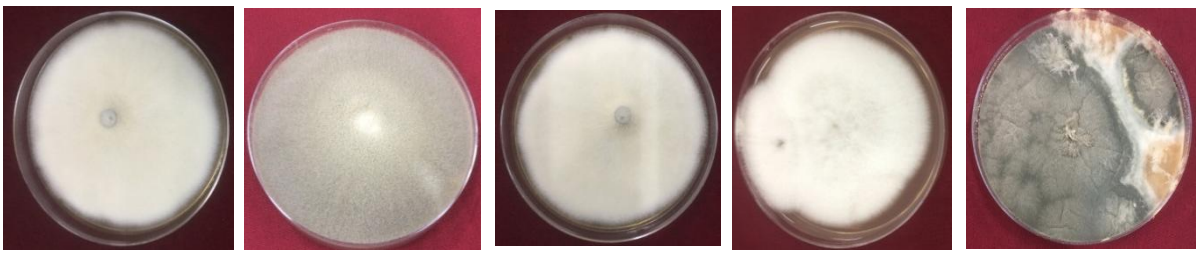
3. RESULTS AND DISCUSSION

3.1 Survey and Isolation of Endophytic Fungi

A survey was conducted in 12 districts of Assam *viz*., Jorhat, Golaghat, Nagaon, Marigaon, Goalpara, Dibrugarh, Tinsukia, Lakhimpur, Dhemaji, Sivsagar, Kamrup and Barpeta and 92 healthy banana root samples were collected from different banana cultivars *viz*., Malbhog, SeniChampa and Jahaji. A total of 55 endophytic fungal isolates were obtained in the present study (Plate1). Isolation of endophytic fungal strains from various monocots and woody plants has been reported by several authors [26-28].

3.2 *In vitro* Efficacy against *M. incognita*

Out of 55 fungal isolates screened, 5 isolates *viz*., BF7, BF27, BF28, EF4 and BF35 recorded 100 per cent inhibition of egg hatching of *M. incognita* with an exposure period of 24, 48 and 72 h at 100 per cent concentration of culture filtrate compared to distilled water. The highest juvenile mortality of *M. incognita* was recorded by 96.33 to 81.33 % in 5 fungal isolates *viz*., BF7, BF27, BF28, EF4 and BF35 at 72h exposure period compared to untreated control (Table 1, Figs.1 & 2). The ovicidal and larvicidal activities against *M. incognita* may be due to parasitization, production of the toxin, secondary metabolites and antibiotics. The findings of [17] were in accordance with the present study where they have reported endophytic fungi inhibited *M. incognita* egg hatching up to 17–26% and increased juvenile mortality of *M. incognita* up to 62–73% compared to control [29]. *Acremonium implicatum* showed increased juvenile mortality of *M. incognita* by 96 per cent compared to control.



EF4 **BF7** **BF27** **BF28** **BF35**

Plate 1. Promising five endophytic fungal isolates

Table 1. *In vitro* efficacy of endophytic fungal isolates against *M. incognita*

S. No.	Endophytic isolates	Number of eggs hatched with exposure period			Number of dead juveniles with exposure period		
		24 h	48 h	72 h	24 h	48 h	72 h
1	EF1	59.33 (7.74)	65.67 (8.13)	70.33 (8.42)	0.00 (0.71)	0.33 (0.91)	1.00 (1.22)
2	EF2	22.33 (4.78)	38.33 (6.23)	46.00 (6.82)	0.00 (0.71)	0.33 (0.91)	2.33 (1.68)
3	EF3	12.67 (3.63)	18.67 (4.38)	28.67 (5.40)	0.00 (0.71)	1.00 (1.22)	2.67 (1.78)
4	EF4	14.00 (3.81)	23.33 (4.88)	34.67 (5.93)	45.67 (6.76)	64.67 (8.04)	81.33 (9.04)
5	EF5	8.33 (2.97)	19.00 (4.42)	24.67 (5.02)	0.00 (0.71)	2.33 (1.68)	4.33 (2.20)
6	EF6	15.67 (4.02)	27.67 (5.31)	37.00 (6.12)	0.00 (0.71)	2.00 (1.58)	4.67 (2.27)

S. No.	Endophytic isolates	Number of eggs hatched with exposure period			Number of dead juveniles with exposure period		
		24 h	48 h	72 h	24 h	48 h	72 h
7	EF7	28.67 (5.40)	33.67 (5.85)	42.67 (6.57)	0.00 (0.71)	4.33 (2.20)	7.67 (2.86)
8	EF8	9.67 (3.19)	20.00 (4.53)	31.67 (5.67)	0.00 (0.71)	4.33[h] (2.20)	11.33 (3.44)
9	EF9	7.67 (2.86)	12.00 (3.54)	18.00 (4.30)	0.00 (0.71)	6.00[g] (2.55)	15.00 (3.94)
10	EF10	10.67 (3.34)	15.00 (3.94)	22.33 (4.78)	0.00 (0.71)	2.00 (2.58)	6.00 (2.55)
11	EF11	22.67 (4.81)	38.33 (6.23)	47.00 (6.89)	0.00 (0.71)	2.00 (2.58)	5.67 (2.48)
12	EF12	29.33 (5.46)	38.00 (6.20)	46.67 (6.87)	0.00 (0.71)	2.00 (2.58)	4.33 (2.20)
13	EF13	17.33 (4.22)	32.00 (5.70)	41.67 (6.49)	0.00 (0.71)	2.33 1.68)	2.67 (1.78)
14	AF1	17.67 (4.26)	35.00 (5.96)	68.00 (8.28)	0.00 (0.71)	1.00 (1.22)	1.67 (1.47)
15	AF2	16.00 (4.06)	26.00 (5.15)	36.00 (6.04)	0.00 (0.71)	0.33 (0.91)	1.00 (1.22)
16	AF3	18.00 (4.30)	28.67 (5.40)	46.33 (6.84)	0.00 (0.71)	0.67 (1.08)	1.33 (1.35)
17	AF4	12.00 (3.54)	14.33 (3.85)	27.00 (5.24)	0.00 (0.71)	0.67 (1.08)	1.33 (1.35)
18	BF1	7.67 (2.86)	18.33 (4.34)	27.33 (5.28)	9.33 (3.14)	21.00 (4.64)	33.67 (5.46)
19	BF2	8.67 (3.03)	19.33 (4.45)	28.33 (5.37)	0.00 (0.71	0.00 (0.71	0.00 (0.71
20	BF3	10.67 (3.34)	21.00 (4.64)	31.33 (5.64)	0.33 (0.91)	1.67 (1.47)	4.33 (2.20)
21	BF4	7.67 (2.86)	16.67 (4.14)	30.67 (5.58)	0.00 (0.71)	0.67 (1.08)	4.33 (2.20)
22	BF5	4.33 (2.20)	15.67 (4.02)	30.33 (5.55)	0.00 (0.71	0.00 (0.71)	0.67 (1.08)
23	BF6	10.33 (3.29)	21.33 (4.67)	30.67 (5.58)	0.00 (0.71)	0.67 (1.08)	2.00 (1.58)
24	BF7	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)	88.00 (9.41)	91.00 (9.57)	96.33 (9.82)
25	BF8	20.67 (4.60)	34.67 (5.93)	68.33 (8.30)	0.33 (0.91)	2.67 (1.78)	7.67 (2.86)
26	BF9	6.67 (2.68)	17.33 (4.22)	26.33 (5.18)	0.00 (0.71	3.33 (1.96)	9.33 (3.14)
27	BF10	18.67 (4.38)	31.67 (5.67)	64.67 (8.07)	0.33 (0.91)	1.33 (1.35)	3.00 (1.87)
28	BF11	14.33 (3.85)	24.67 (5.02)	33.67 (5.85)	0.00 (0.71)	0.00 (0.71)	0.67 (1.08)
29	BF12	13.33 (3.72)	21.67 (4.71)	35.67 (6.01)	12.00 (3.54)	21.00 (4.64)	31.67 (5.67)
30	BF13	5.33 (2.42)	14.33 (3.85)	23.67 (4.92)	5.67 (2.48)	16.33 (4.10)	29.33 (5.46)
31	BF14	4.33 (2.20)	9.33 (3.14)	20.33 (4.56)	0.00 (0.71)	2.00 (1.58)	9.67 (3.19)
32	BF15	7.67 (2.86)	16.33 (4.10)	36.33 (6.07)	0.00 (0.71	0.00 (0.71	0.33 (0.91)
33	BF16	12.33 (3.58)	22.33 (4.78)	31.33 (5.64)	0.33 (0.91)	3.00 (1.87)	9.00 (3.08)

S. No.	Endophytic isolates	Number of eggs hatched with exposure period			Number of dead juveniles with exposure period		
		24 h	48 h	72 h	24 h	48 h	72 h
34	BF17	11.33	18.67	28.33	3.33	11.00	19.33
		(3.44)	(4.38)	(5.37)	(1.96)	(3.39)	(4.45)
35	BF18	18.33	29.33	38.67	4.33	15.67	23.67
		(4.34)	(5.46)	(6.26)	(2.20)	(4.02)	(4.92)
36	BF19	13.33	22.33	32.33	0.00	1.00	2.67
		(3.72)	(4.78)	(5.73)	(0.71	(1.22)	(1.78)
37	BF20	11.67	22.33	33.33	0.00	0.00	0.67
		(3.49)	(4.78)	(5.82)	(0.71	(0.71	(1.08)
38	BF21	12.67	23.67	34.67	5.33	10.33	21.33
		(3.63)	(4.92)	(5.93)	(2.42)	(3.29)	(4.67)
39	BF22	16.67	25.67	34.67	0.00	0.00	0.33
		(4.14)	(5.12)	(5.93)	(0.71	(0.71	(0.91)
40	BF23	3.67	7.33	14.67	0.00	8.67	18.00
		(2.04)	(2.80)	(3.89)	(0.71)	(3.03)	(4.30)
41	BF24	18.67	28.00	37.67	0.00	0.67	4.00
		(4.38)	(5.34)	(6.18)	(0.71	(1.08)	(2.12)
42	BF25	11.33	23.00	33.33	0.00	1.00	4.33
		(3.44)	(4.85)	(5.82)	(0.71	(1.22)	(2.20)
43	BF26	9.33	20.33	29.67	9.67	11.33	28.33
		(3.14)	(4.56)	(5.49)	(3.19)	(3.44)	(5.37)
44	BF27	0.67	1.67[b]	3.67	69.00	81.00	92.67
		(1.08)	(1.47)	(2.04)	(8.31)	(9.03)	(9.62)
45	BF28	0.00	0.00	0.00	78.00	86.00	88.67
		(0.71)	(0.71)	(0.71)	(8.86)	(9.30)	(9.41)
46	BF29	10.67	21.67	30.67	0.00	0.00	0.00
		(3.34)	(4.71)	(5.58)	(0.71	(0.71	(0.71
47	BF30	8.67	19.33	29.33	0.00	2.00	6.67
		(3.03)	(4.45)	(5.46)	(0.71	(1.58)	(2.68)
48	BF31	14.00	16.33	25.33	0.00	1.33	5.00
		(3.81)	(4.10)	(5.08)	(0.71)	(1.35)	(2.35)
49	BF32	11.33	21.33	30.33	0.00	2.00	7.33
		(3.44)	(4.67)	(5.55)	(0.71)	(1.58)	(2.80)
50	BF33	9.00	21.33	31.00	0.00	0.00	0.33
		(3.08)	(4.67)	(5.61)	(0.71	(0.71	(0.91)
51	BF34	13.33	22.33	34.33	0.00	1.33	5.33
		(3.72)	(4.78)	(5.90)	(0.71	(1.35)	(2.12)
52	BF35	1.33	4.67	11.33	16.67	25.67	34.67
		(1.35)	(2.27)	(3.44)	(4.14)	(5.12)	(5.93)
53	BF36	3.67	10.67	19.67	3.67	10.33	18.67
		(2.04)	(3.34)	(4.49)	(2.04)	(3.29)	(4.38)
54	BF37	7.33	16.67	27.67	0.00	1.67	5.00
		(2.80)	(4.14)	(5.31)	(0.71	(1.47)	(2.35)
55	BF38	10.00	20.33	34.33	0.00	0.00	1.00
		(3.24)	(4.56)	(5.90)	(0.71	(0.71	(1.22)
56	Potato dextrose broth	50.33 (7.13)	67.00 (8.22)	95.66 (9.81)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)
57	Distilled water	52.66 (7.29)	73.00 (8.72)	98.66 (9.96)	0.00 (0.71)	0.00 (0.71)	0.00 (0.71)
	S. Ed	0.53	0.65	0.89	0.53	0.64	0.63
	CD (P=0.01)	1.37	1.69	2.31	1.35	1.64	1.61

Values in parenthesis are square root transformed values
CD (P=0.01) indicate that significance of results @ 1 per cent interval

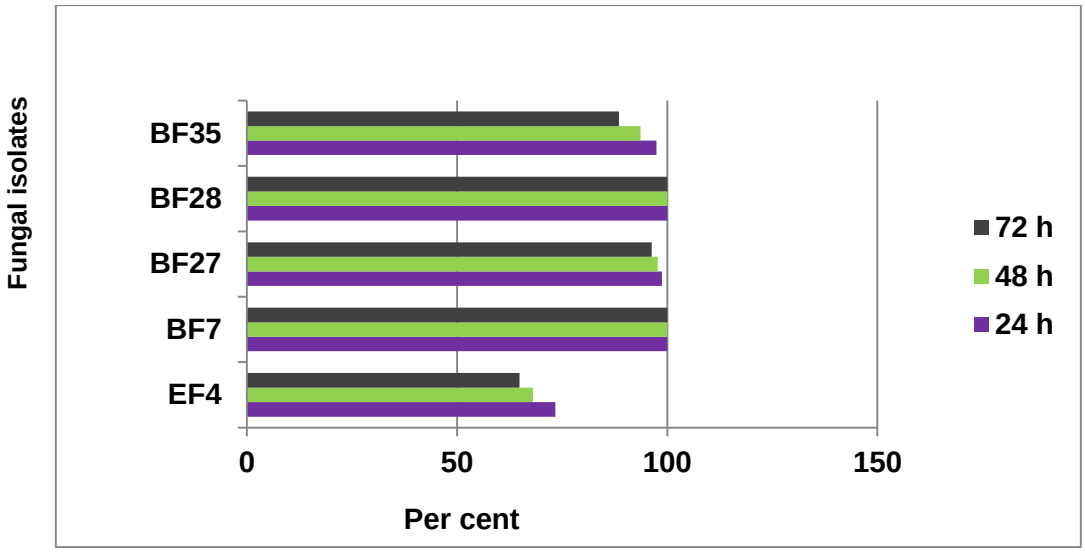


Fig. 1. *In vitro* efficacy of endophytic fungal isolates on inhibition of egg hatching of *M. incognita*

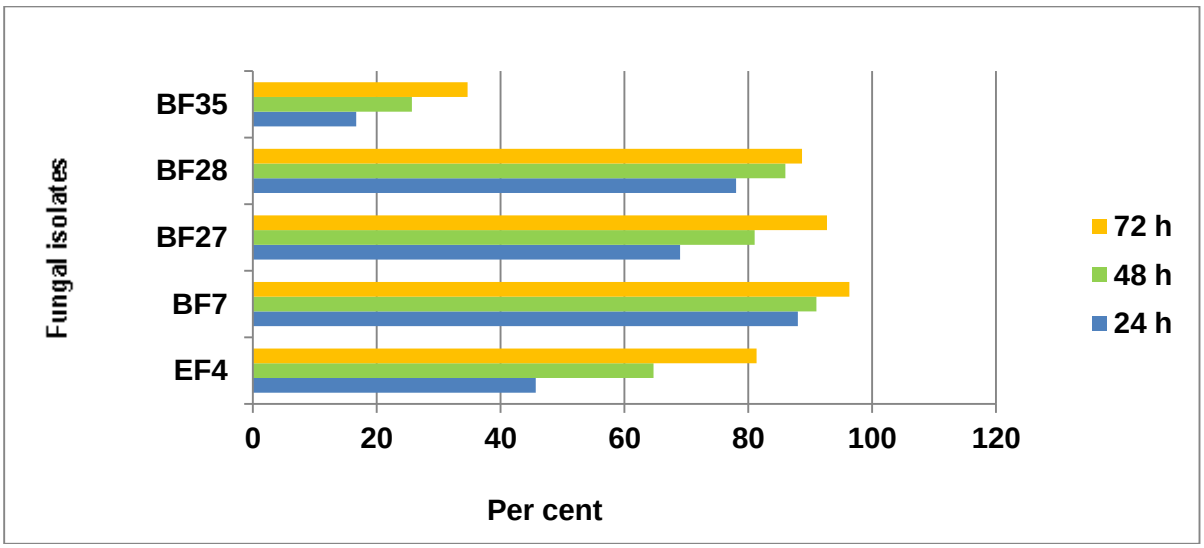


Fig. 2. *In vitro* efficacy of endophytic fungal isolates on juvenile mortality of *M. incognita*

3.3 Growth Promotion Activity

The present results showed that seed treatment with endophytic fungal isolates *viz*., BF7 and BF28 enhanced vigour index of paddy by 62.91 and 47.24%, respectively. Similarly it also increased the germination percentage (82.67; 73.33%), shoot length (20.16; 30.03%) and root length (43.94; 36.58%). Yan *et al*. [30] have indicated that fungal endophytes promoted the growth and health of crop plants. Enhancement of growth may be production of phytohormones like IAA and enhanced availability of nutrients, reduction of ethylene level, production of antibiotics and induced systemic resistance. Antagonistic fungal endophytes improved the resistance and exhibited plant growth promoting rhizobacteria (PGPR) activities [4, 31-34]. The present results were also in accordance with the earlier reports (Fig. 3).

3.4 Pot Culture Evaluation of Fungal Endophytes against *M. incognita*

The best performing five fungal isolates were screened for their nematicidal action against root knot nematode, *M. incognita* in banana based on the results of *in vitro* efficacy and growth

promotion activities. Banana suckers treated with culture filtrate of BF7 isolate recorded significant reduction in number of adult females and number of egg masses by 44.33 and 32.67 %, which was followed by BF28 isolates (53.67; 40.33%). The reduction in soil and root nematode population was also recorded in BF7 treated plants by 39.11 and 36.63%, respectively followed by BF28, which accounted for 33.33 and 28.57 per cent reduction over control, respectively. The lowest root gall index (2.33; 2.67) was registered both in BF7 and BF28 treated banana plants compared to untreated control (5.00) (Figs.4 and 5).

The banana suckers treated with culture filtrates of BF7 and BF28 were significantly reduced the number of adult females, egg masses, root and soil population of *M. incognita* under pot culture conditions. Similar findings were also reported by [35-36]. They reported that endophytic fungi significantly reduced the soil and root population, root gall index compared to untreated control. Waweru *et al*. [33] also found that combined application of *F. oxysporum* and *P. lilacinum* reduced nematode density by 68 per cent; application of *F. oxysporum* and *Bacillus firmus* resulted in significant reduction in *Radopholus similis* population by 86.2 per cent in banana.

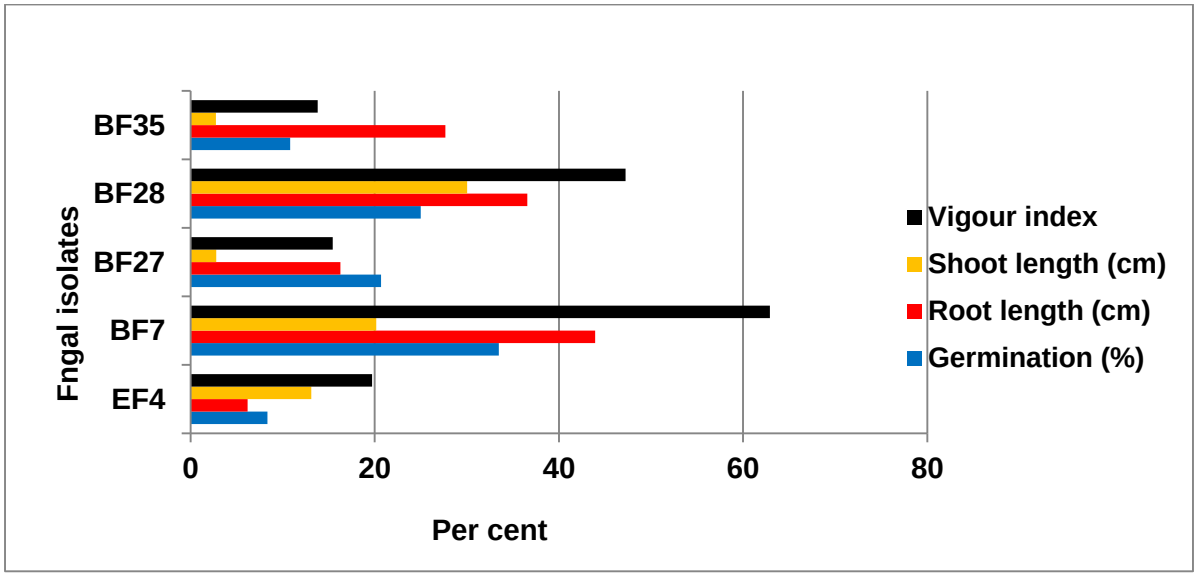


Fig. 3. Growth promotion activity of endophytic fungal isolates in Paddy cv. Co.52 by modified roll towel method

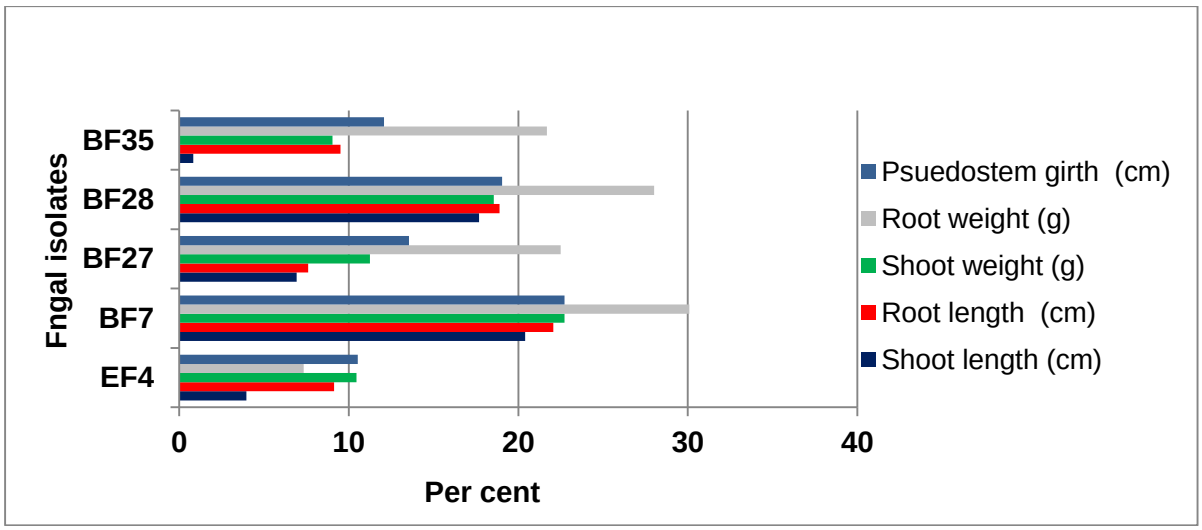


Fig. 4. Influence of endophytic fungal isolates on growth and development of banana under pot culture conditions

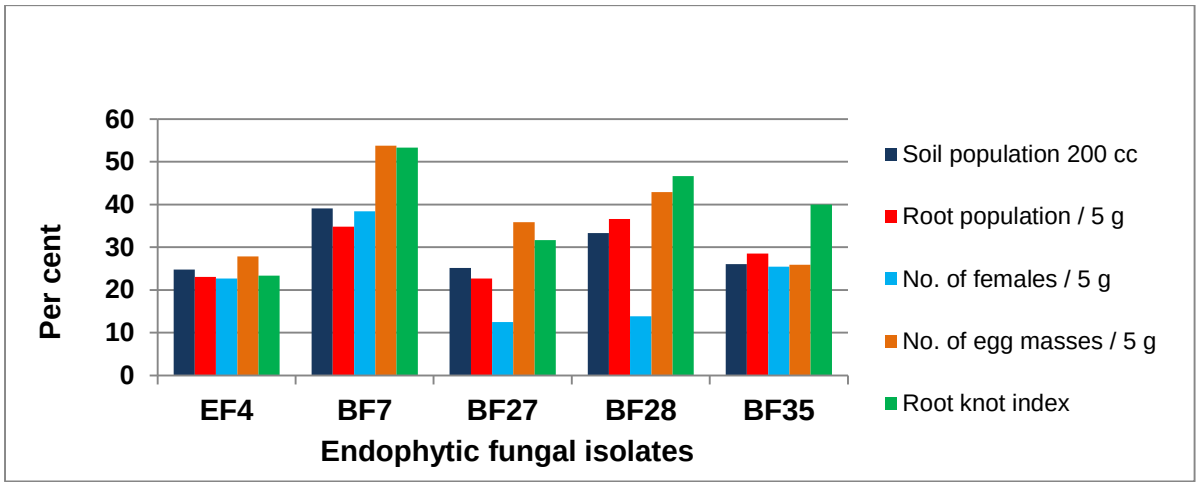
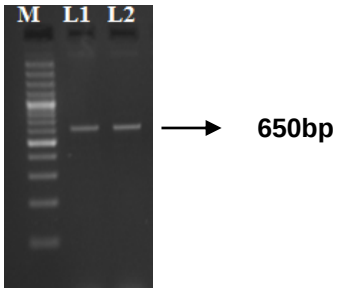


Fig. 5. Effect of endophytic fungal isolates on root knot nematode multiplication under pot culture conditions

The reduction in nematode population might be competition for space and nutrients, premature egg hatching and reduction in viability and mortality of juveniles caused by production metabolites *viz*., chaetoglobosin A, chaetoglobosin B, flavipin, 3-methoxyepicoccone and 4,5,6-trihydroxy-7-methylphthalide by *Chaetomium globosum* against *M. incognita* [37]. The culture filtrate of BF7 significantly increased growth parameters *viz*., shoot length, root length and pseudostem girth by 20.39, 22.06 and 22.73 %, respectively followed by BF28 isolate which was recorded by 17.69, 18.89 and 19.05%, respectively in banana under pot culture conditions. The present results were also in conformity with the earlier reports of Mingot-Ureta *et al*. [38] found that the endophytic fungal strains significantly increased the growth parameters *viz*., pseudostem height, girth and number of leaves in banana plants under glasshouse conditions.

3.5 PCR- 18S rRNA of ITS Region Sequencing

The genomic DNA was extracted from two fungal isolates and amplified the full length of ITS region about ≈ 650bp (Plate1). The gel purified PCR products were sequenced in both directions and the orientation of the sequence was corrected by Bioedit software. The data were subjected to comparison with other 18S rRNA of ITS region available in the NCBI gene bank database. In sequence analysis, endophytic fungal isolates *viz*., BF7and BF28 revealed 100 % sequence similarity with non- pathogenic *F. oxysporum.* The similar results were also reported earlier by [39,40].The isolates BF7 and BF28 form clusters with the non-pathogenic isolates indicated that the isolated strain in the present study also non-pathogenic (Plate 2 and 3). An out-group isolate, *Fusarium proliferatum* was used to distinguish between pathogenic and non-pathogenic strains.



M 100 bp marker
Lane 1- BF7
Lane 2- BF28

Plate 2. Polymer chain reaction amplification on ITS region of promising endophytic fungal isolates

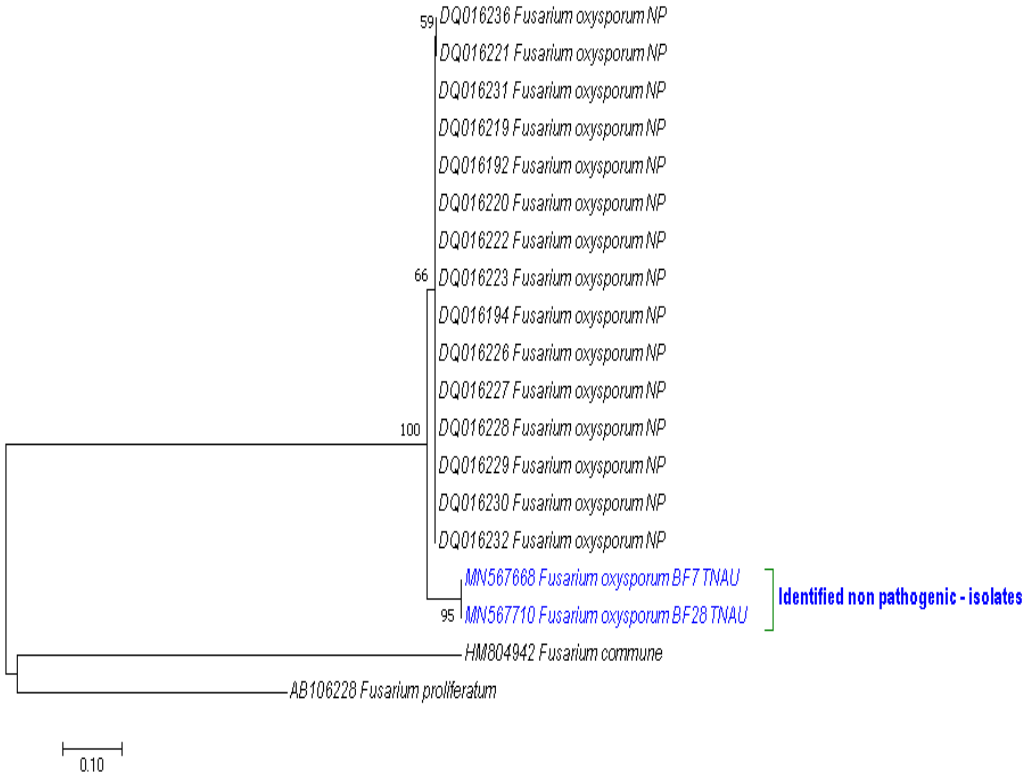


Plate 3. Phylogenetic relationship of endophytic fungal based on 18S rRNA-ITS region of gene sequences

The present results were also in conformity with the earlier reports of Stewart *et al*. [41] found that phylogenetic relationship showed that the isolates of non-pathogenic *F. oxysporum* with biological control activity were grouped into one by using bootstrap values and neighbour - joining method.

4. CONCLUSION

Endophytic fungi isolates play a significant role in enhancing the plant growth and reducing population density and multiplication of root knot nematode, *M. incognita* in banana was confirmed in the present study. Further, investigations on tripartite relationship between nematode, plant and endophytes has to be carried out by transcriptome analysis to understand specific gene regulations. This study will help in understanding of biocontrol potential of fungal endophytes against nematodes.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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