Management of Root Knot Nematode *Meloidogyne incognita* in Banana (Cv. Robusta) through Biocontrol Agents and Neem Cake

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**ABSTRACT**

A pot culture experiment was performed to study the effect of native promising biocontrol agents and neem cake separately and in combination against root-knot nematode *Meloidogyne incognita* infecting banana cv. Robusta during 2016-2017. Treatments treated with bioagents and neem cake enhanced plant growth and root characters and suppressed root gall development in banana plant as compared to nematode alone and untreated control treatments. Among the various treatments, the combined application of *Pseudomonas fluorescens* @ 10 g/plant + *Trichoderma viride* @ 10 gm/plant + neem cake @ 100 gm/plant resulted in maximum increase of plant height (38.8 cm), number of leaves (7), pseudostem girth (15 cm), root length (25.6 cm), highest number of healthy roots (17.0), poor number of infected roots (3.3), lowest root gall index (1 scale) and significantly reduced root-knot nematode population both soil (55.0 nos) and roots (90.0 nos). The application of single bioagent either *P. fluorescens* @ 10 gm/plant or *T. viride* @ 10 gm/plant along with neem cake were also recorded highest plant growth parameters and poor nematode infestation when compared to nematode alone treated control plant.

**Key words:** Banana, Management, *Meloidogyne incognita*, Neem cake, *P. fluorescens*, *T. viride*.

**INTRODUCTION**

Banana is one of the important fruit crops in India cultivated over 0.83 million ha, constituting about 44.3 per cent of total world fruit production. In recent years, the intensive cultivation of banana on a commercial scale is threatened by several pests and diseases. Among the major biotic stresses, plant parasitic nematodes pose a daunting challenge for banana production. Among the plant parasitic nematodes, the root-knot nematode *Meloidogyne incognita* is the most important pest of several Agricultural and Horticultural crops which caused severe yield losses. Yield losses due to the root knot-nematode was previously worked out by earlier researchers in different crops. For example in banana 30.9 per cent (Jonathon and Rajendran, 2000), in Pomegranate 17.3 per cent (Singh et al, 2019) and betelvine 26-38 per cent (Jayant Bhatt and Indira Vadhara, 2004). Jain et al, (2007) also reported that the estimated crop yield losses to the extent of 16.67%, 18.20%, 21.35%, 14.10%, 27.21%, 10.54% and 12.85% in brinjal, cucurbits, jute, okra, tomato, rice and chilly respectively in India.

Microorganisms such as *Pseudomonas* spp., *Bacillus* spp., *Pasteuria* spp., *Paecilomyces lilacinus*, *Verticillium* spp., *Trichoderma* spp. and Vesicular Arbuscular Mycorrhizae fungi have been recognized as most important candidates for reducing different plant parasitic nematodes in several crops (Cannayane and Rajendran, 2002). Nowadays the use of bioagents with neem cake is considered as one of the alternative approaches for the management of nematode disease as it is safe to environment, no health hazards to human beings, economic and easily available to farmers in comparison to chemicals. Hence, an investigation was undertaken for the management of root-knot nematode *M. incognita* on banana using the promising native biocontrol agents such as *P. fluorescens*, *T. viride* and neem cake separately and in combination.

**MATERIALS AND METHODS**

**Isolation and formulation of Bioagents**

Native soil sample were collected from rhizosphere of banana plants to isolate biocontrol agents, *Pseudomonas fluorescens* and *Trichoderma viride* by serial dilution plate technique. 0.1 ml of each 10^{-5} and 10^{-6} dilution was pipetted out into serial Petri plates containing King’s B medium (King et al., 1954) for *P. fluorescens*, Potato Dextrose Agar (PDA)
and Malt extract agar (Gams and Bisset, 1998) for T. viride. The confirmation of P. fluorescens using various morphological and biochemical key's given at Beary's manual of Bacteriology. The PDA and Malt extract agar containing plates were incubated at 25°C for 7 days. T. viride colonies were identified according to the identification key based on branching of conidiophores, shape of phialides, emergence of phialides and spore characters (Gams and Bisset, 1998).

The confirmative bioagents were formulated in purified talc powder (sterilized at 105°C for 12h) with calcium carbonate 15g and carboxyl methyl cellulose (CMC) 10g/kg. (Vidhyasekaran and Muthamilan, 1995). At the time of application, the population of P. fluorescens in talc formulation was 2×10^8 cfu/g and T. viride 2×10^8 cfu/g. These were tested separately and in combination with and without neem cake against root-knot nematode M. incognita infesting banana under shade net condition.

Pure culture of Meloidogyne incognita

Egg masses of root-knot nematode, M. incognita were collected from pure culture maintained on coleus plants. These egg masses were transferred into a beaker containing tap water. The top of the beaker was closed with a piece of polythene sheet with small holes for aeration. This was incubated at room temperature for 3-4 days. The water at the top was removed and then fresh water was added and aerated every day. After three days, freshly hatched juveniles (J2) of M. incognita were collected and used as a source of inoculum. To estimate the number of J2 used for treatments, J2 individuals obtained per ml were counted in triplicates and later averaged. Distilled water was used to adjust to 2000 J2 per treatment. The collection and preparation of J2 was described by Sundararaju and Kiruthika, (2009).

Experimental details

The pot culture experiment was conducted at Post Graduate and Research Department of Zoology, Government Arts College (Autonomous), Karur-639 005, TamilNadu, India, during 2016-2017. Uniform size banana suckers (cv.Robusta) were planted in pots (size: 30 cm width and 60 cm height) containing sterilized soil mixture (Red soil: sand: FYM) in the ratio of 2:1:1. After one month of planting freshly hatched J2 of M. incognita were inoculated at the rate of 2000 J2/plant except uninoculated control. After 1 week of inoculation the bioagent and neem cake were applied in the treatments given below.

T1. Neem cake @ 100 gm/plant
T2. Pseudomonas fluorescens @ 10 gm/plant
T3. Trichoderma viride @ 10 gm/plant
T4. Pseudomonas fluorescens @ 10 gm/plant + Neem cake @ 100 gm/plant
T5. Trichoderma viride @ 10 gm/plant + Neem cake @ 100 gm/plant
T6. Pseudomonas fluorescens @ 10 gm/plant + Trichoderma viride @ 10 gm/plant + Neem cake @ 100 gm/plant
T7. Nematode alone

T8. Untreated Control (Check)

Each treatment was replicated thrice and arranged in randomized block design (RBD). All the treated plants were watered periodically. The experiment was terminated after 120 days after planting. At the time of termination, the plants were carefully uprooted with intact root system. The soil and root samples were collected for estimating final nematode population. Observations were recorded on plant height (cm), number of leaves, Pseudostem girth (cm), root length (cm), root weight (g), number of infected roots, number of healthy roots and root-knot index. The root galling was assessed by examining the roots on the outside by visual observation of the whole root system based on the percentage of galled roots. The percentage was converted to a scale of 0-5 as suggested by Sundararaju and Kiruthika, (2009). After this, roots were cut into small pieces, mixed thoroughly and samples of 5gm each were stained in boiling acid fuschin-lacto phenol. These were blended and nematode populations were assessed. Soil samples (250cc) were also collected from each pot for estimating the nematode population. Nematode population from soil was estimated by using Cobb’s sieving and decanting method (Cobb’s 1918) followed by modified Baermann’s funnel method (Christie and Perry, 1951).

The data were subjected to statistical scrutiny by analysis of variance, standard error of deviation and co-efficient of variance using AgRes statistical software (1994, Pascal International Software Solutions). Critical difference at P=0.05 % were calculated by the statistical method developed by Panse and Sukhatme (1978).

RESULTS AND DISCUSSION

Significant increases in plant growth and root indices were observed in all the treatments over nematode alone inoculated treatment and untreated control. The combined application of neem cake along with bio-agents P. fluorescens, T. viride against M. incognita resulted that significant increase in plant height (38.8 cm), number of leaves (7) and pseudostem girth (15.0 cm), root length (25.6 cm), root weight (37.8 gms), total number of roots (20.3) number of healthy roots (17.0) number of infected roots (3.3), minimum root gall index (1) and nematode population of 55.0 from soils and 90.0 from roots was recorded over nematode alone treated control (Table 1 and 2). The present results were confirmative with earlier workers Pandey et al. (2005) who reported an eco-friendly management of root-knot nematode and Fusarium wilt disease infecting chickpea by integrating fungal bio-agents and oil cakes. The application of organic amendments and use of biocontrol agents on plant parasitic nematodes were gaining much importance (Kalaiarasan et al. 2006). Konsam et al. (2013) have reported that application of jatropha cake, neem cake and castor cake could enhance growth and yield of cucumber by reducing nematode population.

The significant increase in plant height (35.0 cm), number of leaves (6) and pseudostem girth (13.6 cm), root
length (23.0), root weight (36.2 gm), total number of roots (18.0), number of healthy roots (14.0) and number of infected roots (4.0), medium root girth index (3) and nematode population of 175.3 from soils and 302.0 from roots were recorded from plants treated with neem cake and P. fluorescens over nematode alone treated control (Table 1 and 2). The plant growth promoting rhizobacteria such as P. fluorescens would effective against root-knot nematode, Meloidogyne incognita in banana (Jonathan et al. 2006). Nama and Sharma (2017) further reported that P. fluorescens could increase the plant growth by reducing nematode population. Kar et al. (2018) also reported that the application of P. fluorescens @ 20 g m⁻² + neem cake @ 100 g m⁻² increased the cowpea production by reducing nematode population.

Increase in plant height (34.3 cm), number of leaves (5) and pseudostem girth (13.0 cm), root length (22.1 cm), root weight (34.8 gm), total number of roots (17.3), number of healthy roots (13.0), number of infected roots (4.3), minimum root girth index (2) and nematode population of 230.0 from soils and 419.0 were recorded from plants treated with neem cake and bio-agent T.viride over nematode alone treated control. The rest of other treatments were superior over nematode alone and untreated control treatment plots (Table 1 and 2). The combined application of T.viride and neem cake reduced the multiplication rate of nematodes (Farooq Azam et al., 2007). The neem cake and T.viride applications supported the growth in addition to their adverse effects on M. incognita which ultimately led to improved plant growth characteristics (Sunil Kumar and Anju Khanna, 2006). The above results were confirmed by the workers of Shanthi and Rajendran, (2006) who reported that the application of P. fluorescens at 20 g/plant reduced the populations of Radopholus similis, Pratylenchus coffeae and Helicotylenchus multicinctus by 48.7, 46.3 and 44.3%, respectively in banana. P. fluorescens at 10⁶ CFU/ml decreased Meloidogyne javanica infections, compared to the control. Also, it was able to cause destruction of nematode egg mass matrix and significantly decreased nematode egg hatching level (Norabadi et al., 2014). Combined applications of bioagents were more effective in reducing the disease incidence than the

### Table 1: Plant growth parameters on the management of M.incognita in banana through promising biocontrol agents and neem cake.

| Treatments                                           | Plant Height (cm) | No. of Leaves | Pseudostem girth (cm) | Root length (cm) | Root weight (g) |
|-------------------------------------------------------|-------------------|---------------|-----------------------|------------------|-----------------|
| T₁ Neem cake                                         | 30.0              | 4             | 11.8                  | 21.0             | 32.2            |
| T₂ Pseudomonas fluorescens                           | 32.6              | 5             | 12.6                  | 21.8             | 32.0            |
| T₃ Trichoderma viride                                | 31.5              | 4             | 12.0                  | 21.2             | 33.0            |
| T₄ Pseudomonas fluorescens + Neem cake                | 35.0              | 6             | 13.6                  | 23.0             | 33.8            |
| T₅ Trichoderma viride + Neem cake                     | 34.5              | 5             | 13.0                  | 22.1             | 32.6            |
| T₆ Pseudomonas fluorescens + Trichoderma viride       | 36.6              | 6             | 14.3                  | 23.5             | 35.0            |
| T₇ Pseudomonas fluorescens + Trichoderma viride + Neem cake | 38.8              | 7             | 15.0                  | 25.6             | 35.6            |
| T₈ Nematode alone                                    | 23.0              | 3             | 6.0                   | 16.0             | 25.0            |
| T₉ Untreated Control (Check)                         | 29.5              | 4             | 11.0                  | 18.4             | 30.0            |

### Table 2: Plant root parameters and nematode population on the management of M.incognita in banana through promising biocontrol agents and Neem cake.

| Treatments                                           | Total No. of roots | Number of healthy roots | Number of infected roots | Root-knot index (0-5) | Nematode populations |
|-------------------------------------------------------|--------------------|-------------------------|--------------------------|-----------------------|----------------------|
| T₁ Neem cake                                         | 15.5               | 10.0                    | 5.5                      | 3                     | 380.0                |
| T₂ Pseudomonas fluorescens                           | 16.8               | 12.1                    | 4.7                      | 3                     | 300.0                |
| T₃ Trichoderma viride                                | 16.0               | 11.0                    | 5.0                      | 2                     | 360.0                |
| T₄ Pseudomonas fluorescens + Neem cake                | 18.0               | 14.0                    | 4.0                      | 2                     | 175.3                |
| T₅ Trichoderma viride + Neem cake                     | 17.3               | 13.0                    | 4.3                      | 3                     | 230.0                |
| T₆ Pseudomonas fluorescens + Trichoderma viride       | 19.6               | 16.0                    | 3.6                      | 2                     | 103.6                |
| T₇ Pseudomonas fluorescens + Trichoderma viride + Neem cake | 20.3               | 17.0                    | 3.3                      | 1                     | 55.0                 |
| T₈ Nematode alone                                    | 10.2               | 2.2                     | 8.0                      | 5                     | 490.0                |
| T₉ Untreated Control (Check)                         | 14.0               | 14.0                    | 0.0                      | 0                     | 0                    |

SEd | 0.25 | 1.18 | 1.66 | 1.57 | 53.32 |

CD (P<0.05) | 2.65 | 2.51 | 3.51 | 3.34 | 113.05 |

CV% | 4.74 | 29.72 | 16.70 | 8.92 | 140.81 |
individual treatments. *T. harzianum* + *P. fluorescens* + *Bacillus subtilis* minimized the nematode population and increased plant growth parameters in Ginger (Dohroo and Meenu Gupta, 2014).

**CONCLUSION**

From the results of the present study it is inferred that though the individual application of *P. fluorescens* and neem cake is effective in the control of root-knot nematode *M. incognita*, the combined application of *P. fluorescens* @10 g/plant or *T. viride* @10 g/plant with neem cake @ 100 g/plant is more effective in controlling the root-knot nematode but also increasing the growth of the plant.

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