Bio-management of rice root-knot nematode, *M. graminicola* by using various organic amendments and bio-control agents on rice nursery

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**ABSTRACT**

A screen house experiment was conducted during kharif season, 2020-21 to know the effect of organic amendments and bio-agents (alone and in combination) in comparison with chemical on the population of rice root-knot nematode (*Meloidogyne graminicola*) in rice. Nursery was grown in 5 kg soil capacity earthen pan filled with infested soil having initial nematode population 285 J2/200 cc soil. Seeds of the rice (Variety- Pusa 1121) were soaked in tap water for 24 h and the sprouted seeds were sown in pots. Hundred seeds of rice were sown in each pot and each treatment was replicated four times. Organic amendments (neem cake and FYM) and bio-agents (*Pseudomonas fluorescens*, *Purpureocillium lilacinum* and *Trichoderma viride*) were added 10 days before sowing. Weighted amount of bio-agents was mixed in neem cake and FYM for enrichment with 7-10 days waiting period. Carbofuran (Furadan 3G) was added at time of sowing. The earthen pots without organic amendments, bio-agents and chemical were treated as control. Observations on plant growth parameters (seedling length in cm fresh and dry seedling weight in g) and nematode multiplication (number of galls/20 seedling, number of eggs/20 seedling and final nematode population in the soil/200 cc soil) were made at transplanting time. The plant parameters were maximum and significantly highest in treated nursery (neem cake @ 50 g/pot + *Pseudomonas fluorescens* @ 50 g/pot) under screen house conditions. Nematode reproduction and multiplication parameters such as number of galls/seedling, number of eggs/seedling and final nematode population were significantly reduced in neem cake @ 50 g/pot + *Pseudomonas fluorescens* @ 50 g/pot.

**Introduction**

Rice (*Oryza sativa* L.) belongs to the Poaceae family and classified as semi-aquatic crop plants that thrive in a variety of soil and water conditions. Approximately, 90 per cent of the world’s rice is grown and consumed in Asia. In India, it was cultivated from ancient time and ranked first in area and second in production after China. Annually, the country produces approx. 175.58 million tonnes of rice (FAOSTAT, 2018). Rice is cultivated in almost all the states of country and among which West Bengal is the highest in rice production and Tamil Nadu has first place in productivity. As rice is grown under various conditions so it is affected by a number of abiotic and biotic factors. Among biotic factors, plant parasitic nematodes (PPNs) proved most damaging pathogens (Jain *et al.*, 2012)
which causes 16-32 per cent yield loss under irrigated and 11-73 percent under flooded conditions in India (Tian et al., 2018). Due to these tiny worms on rice, annually estimated globally yield loss ranges from 10-25% (Bridge et al., 2005). Among PPNs, four major nematode species of rice crop are taken into account i.e. *Meloidogyne graminicola*, *Aphelenchoides besseyi*, *Ditylenchus angustus* and *Heterodera oryzicola* that caused combined yield loss to be estimated 10.50 per cent (Jain et al., 2007). However, rice root-knot nematode, *M. graminicola* has become the most destructive pest and serious problem in major rice producing countries of the world including in India (Jain et al., 2012). The diagnostic symptom of *M. graminicola* affected rice plants are less vigor, stunting growth, yellowing (nurseries and main field), production of poorly filled kernels and late maturity of crop. The main problem of this pest is seen particularly in nursery where flooding is intermitted. Since, the initial infection starts from the nursery, the management of this nematode in rice becomes most important. Various effective strategies should be applied in preventing the spread of *M. graminicola* from infected areas to uninfected areas by infected seedlings. Among these preventive or management methods, chemical control should be avoided due to their indiscriminate use which enhance the problem of resistance and risk to the environment. So, there is current need of the adoption of eco-friendly management approaches like organic amendments particularly deoiled cakes and use of effective bio-control agents which can mostly used in rice nursery application. Therefore in present study, we tried to define the effects of integrated management practices on rice root-knot nematode, *M. graminicola* using organic amendments and bio-agents under rice nursery conditions.

**Material and Methods**

The present study was conducted under screen house conditions to know the effect of organic amendments, bio-agents (alone and in combination) by comparing with chemical on the population of rice root-knot nematode in rice. Nursery were grown in 5 kg soil capacity earthen pan filled with infested soil having initial nematode population 285 J2/200 cc soil. Seeds of the rice (Variety - Pusa 1121) were soaked in tap water for 24 h and the sprouted seeds were sown in pots. Hundred seeds were sown in each pan and every treatment was replicated four times. Organic amendments (neem cake and FYM) and bio-agents (*Pseudomonas fluorescens*, *Purpureocillium lilacinum* and *Trichoderma viride*) were added ten days before sowing. Weighted amount of bio-agents was mixed in neem cake and FYM for enrichment and kept for 7-10 days as waiting period for proper multiplication of bio-agents in organic amendments. Carbofuran (Furadan 3G) was added at time of sowing. The earthen pans without organic amendments, bio-agents and chemical were treated as control. After thirty-five days of sowing, all the plants were uprooted carefully. The roots of plants were retrieved carefully and kept under running tap water to clear it from adhering soil particles and recorded the observations such as plant growth parameters (seedling length in cm, fresh and dry seedling weight in g) and nematode multiplication factors (number of galls/20 seedling, number of eggs/20 seedling and final nematode population in the soil/200 cc soil). For recording final soil population, soil from each pan after depotting was analyzed by Cobb’s Sieving and Decanting technique (Cobb, 1918 and Schnidler, 1961) and nematode were extracted by Modified Baermann’s Funnel technique (MBFT).

**Treatment details:**

| Treatment | Description |
|-----------|-------------|
| T1        | Farm yard manure @ 50 g/pot |
| T2        | Neem cake @ 50 g/pot |
| T3        | T1 enriched with *Pseudomonas fluorescens* @ 50 g/pot |
| T4        | T1 enriched with *Purpureocillium lilacinum* @ 50 g/pot |
| T5        | T1 enriched with *Trichoderma viride* @ 50 g/pot |
| T6        | T2 enriched with *Pseudomonas fluorescens* @ 50 g/pot |
| T7        | T2 enriched with *Purpureocillium lilacinum* @ 50 g/pot |
| T8        | T2 enriched with *Trichoderma viride* @ 50 g/pot |
| T9        | Carbofuran (Furadan 3G) @ 200 mg/pot (chemical check) |
| T10       | Untreated control |

**Statistical analysis:**

The data obtained in the experiment was analyzed by complete randomized design (CRD).
Results and Discussion
Generally, the infestation of *M. graminicola* is suppressed by various management components viz., organic amendments, bio-control agents, chemical control etc. The present investigation was on integrated management of *M. graminicola* using organic amendments and bio-agents on rice nursery. After thirty-five days of sowing, observations were recorded on plant growth parameters and nematode reproduction and multiplication factors.

Maximum and significantly higher seedling length (66.1 cm) was recorded in neem cake enriched with *Pseudomonas fluorescens* @ 50 g/pot over untreated check followed by FYM enriched with *Trichoderma viride* @ 50 g/pot (63.9 cm). This was statistically at par with that of Furadan 3G (carbofuran) @ 200 mg/pot (Figure 1). The minimum seedling height (41.1 cm) was observed in untreated check. However, all the treatments significantly increased the seedling height as compared to untreated check except FYM @ 50 g/pot. Between organic amendments higher seedling height was obtained in neem cake followed FYM. Maximum and significantly higher fresh (4.45 g) and dry seedling weight (1.29 g) was observed in plants treated with neem cake enriched with *P. fluorescens* @ 50 g/pot. This was statistically at par with that of FYM enriched with *T. viride* @ 50 g/pot and carbofuran @ 200 mg/pot. However, all the treatments significantly increased the fresh and dry seedling weight as compared to untreated check except individual application of FYM @ 50 g/pot. The findings present study confirms the results of Bansal et al. (2005). Similarly, Kumar (2019) who reported that soil application of neem cake @ 50 g/pot + *P. fluorescens* @ 50 g/pot had significantly higher seedling growth of rice under nursery conditions as compared to untreated check (Table 1). These results are in conformity with those of Anitha and Rajendran (2005) who revealed that the integration of *P. fluorescens* at 2.5 kg/ha, neem cake at 1 t/ha and carbofuran at 1 kg a.i./ha was highly effective in improving rice plant growth parameters in the nursery as well as in main field. Shukla and Chand (2018) also reported that among the various botanicals neem cake @ 15 g/kg soil proved more effective against *M. graminicola* under pot conditions and significantly increase the plant growth parameters and reduce the nematode parameters as compared to botanicals.

In the present investigation, combined soil application of different organic amendments and bio-agents on rice nursery were used, among which neem cake with *P. fluorescens* was superior as compared to other treatments. Significantly higher seedling growth was also recorded in pre sowing application of with neem cake enriched with *P. fluorescens* @ 50 g/pot followed by FYM enriched with *T. viride* @ 50 g/pot. In individual application organic amendments, maximum seedling growth was found in neem cake @ 50 g/pot than FYM @ 50 g/pot. According to Pankaj et al. (2010), use of carbofuran as soil application considerably decreased galling of rice root-knot nematode at 1 kg/ha or above, at 2 kg dose causing the largest reduction in galling (82%). In the year 2013, Mukesh and Sobita also reported that the neem leaf extract significantly increased the plants growth parameters and reduced number of galls in different concentration.

Minimum and significantly lowest number of galls/seedling (29.00) was observed in neem cake enriched with *P. fluorescens* @ 50 g/pot over untreated check. This was statistically at par with that of neem cake enriched with *P. lilacinum* @ 50 g/pot (33.50). The maximum number of galls/seedling (155.75) was however observed in untreated check. Between organic amendments
Table 1. Effect of various treatments on the plant growth parameter of rice infested with *Meloidogyne graminicola*

| Sr. No. | Treatments                                      | Seedling height (cm) | Fresh seedling weight (g) | Dry seedling weight (g) |
|---------|------------------------------------------------|----------------------|---------------------------|-------------------------|
| T1      | Farm yard manure @ 50 g/pot                    | 47.6                 | 2.83                      | 0.82                    |
| T2      | Neem cake @ 50g/pot                            | 51.6                 | 3.25                      | 0.95                    |
| T3      | FYM enriched with *Pseudomonas fluorescens* @ 50 g/pot | 56.4                 | 3.69                      | 1.07                    |
| T4      | FYM enriched with *Purpureocillium lilacinum* @ 50 g/pot | 54.7                 | 3.44                      | 1.00                    |
| T5      | FYM enriched with *Trichoderma viride* @ 50 g/pot | 63.9                 | 4.27                      | 1.24                    |
| T6      | Neem cake enriched with *P. fluorescens* @ 50 g/pot | 66.1                 | 4.45                      | 1.29                    |
| T7      | Neem cake enriched with *P. lilacinum* @ 50 g/pot | 62.2                 | 4.09                      | 1.19                    |
| T8      | Neem cake enriched with *T. viride* @ 50 g/pot  | 61.3                 | 4.15                      | 1.21                    |
| T9      | Carbofuran (Furadan 3G) @ 200 mg/pot (chemical check) | 60.7                 | 3.96                      | 1.15                    |
| T10     | Untreated control                              | 41.1                 | 2.48                      | 0.77                    |
| C.D. at 5%|                                                | 8.3                  | 0.53                      | 0.16                    |

Date of sowing: 17/07/2020; Date of termination: 23/08/2020

Table 2. Effect of various treatments on reproduction and multiplication of *Meloidogyne graminicola* on rice

| Sr. No. | Treatments                                      | Number of galls/seeding | Number of eggs/seeding | Final nematode population in the soil (200 cc soil) |
|---------|------------------------------------------------|-------------------------|------------------------|---------------------------------------------------|
| T1      | Farm yard manure @ 50 g/pot                    | 137.75 (11.78)          | 15525.00 (124.56)      | 246.25 (15.71)                                    |
| T2      | Neem cake @ 50g/pot                            | 131.00 (11.48)          | 11162.50 (105.64)      | 217.50 (14.74)                                    |
| T3      | FYM enriched with *Pseudomonas fluorescens* @ 50 g/pot | 95.50 (9.81)           | 6800.00 (81.80)        | 192.50 (13.86)                                    |
| T4      | FYM enriched with *Purpureocillium lilacinum* @ 50 g/pot | 126.50 (11.29)         | 8792.50 (93.71)        | 198.75 (14.10)                                    |
| T5      | FYM enriched with *Trichoderma viride* @ 50 g/pot | 65.25 (8.11)           | 6570.00 (80.92)        | 187.50 (13.71)                                    |
| T6      | Neem cake enriched with *P. fluorescens* @ 50 g/pot | 29.00 (5.32)           | 1397.50 (36.30)        | 125.00 (11.13)                                    |
| T7      | Neem cake enriched with *P. lilacinum* @ 50 g/pot | 33.50 (5.69)           | 1830.00 (42.33)        | 165.00 (12.38)                                    |
| T8      | Neem cake enriched with *T. viride* @ 50 g/pot  | 34.50 (5.79)           | 1877.50 (41.12)        | 171.25 (13.10)                                    |
| T9      | Carbofuran (Furadan 3G) @ 200 mg/pot (chemical check) | 35.00 (5.86)           | 1907.50 (42.90)        | 172.50 (13.16)                                    |
| T10     | Untreated control                              | 155.75 (12.52)         | 20327.50 (142.41)      | 311.25 (17.13)                                    |
| C.D. at 5%|                                                | (1.58)                | (12.43)                | (3.37)                                             |

Figures in parentheses are √ n transformed value

Date of sowing: 17/07/2020; Date of termination: 23/08/2020
lowest number of galls/seedling was obtained in neem cake than FYM. Minimum and significantly lowest number of eggs/seedling (1397.50) and final nematode population (125.00) was observed in neem cake enriched with \textit{P. fluorescens} @ 50 g/pot over untreated check and this was statistically at par with that of neem cake enriched with \textit{P. lilacinum} @ 50 g/pot. The maximum number of eggs/seedling (20327.50) was however observed in untreated check. Ammonia released during decomposition of the different organic amendments, which are poison to PPNs including root knot nematodes (Mian et al., 1982; Mian and Rodriguez-Kabana, 1982) due to which it may be partially involved in suppression \textit{M. graminicola} and reduced root galling. Similar findings were also observed by Kumar (2019) who concluded that integration of neem cake with \textit{P. fluorescens} under nursery conditions significantly reduced the nematode reproduction and multiplication parameters (Table 2).

Similar results were recorded by Seenivasan et al. (2012) who observed that talc based formulation of \textit{P. fluorescens} and \textit{P. lilacinum} significantly reduced the root invasion and soil population of nematode but \textit{P. fluorescens} was most effective when applied as seed cum soil application and seed treatment alone. Panigrahi and Mishra (1995) also reported that carbofuran significantly reduced galling and also buildup low population of \textit{M. graminicola} when with seedlings in fields planted treated with carbofuran @ 1 kg a.i. per ha. The results of this study is not confirmatory with Devi et al. (2019) who revealed that castor cake @ 10 g/kg soil was found best in improving plant growth parameters and reducing nematode parameters as compared to other organic amendments (neem cake and mustard cake) @5 and 10g/kg against this nematode in rice nursery. This may be due to some ecological and environmental factors. Dangal et al. (2008) also observed number of J2 and plant growth parameters were non-significant in different organic amendments including neem leaves (\textit{Azadirachta indica}) when applied @ 1, 2 and 3 t/ha against this nematode in direct seeded rice.

**Conclusion**

The current study is more realistic because nematode invasion caused significant damage to rice nurseries. Under screen house conditions, rice growth metrics were maximum and significantly higher in treated nursery (neem cake @ 50 g/pot + \textit{Pseudomonas fluorescens} @ 50 g/pot). Nematode reproduction and multiplication parameters such as the number of galls per seedling, the number of eggs per seedling, and the final nematode population were all reduced dramatically.

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**Conflict of interest**

The authors declare that they have no conflict of interest.

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