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Management of rice root-knot nematode, *Meloidogyne graminicola* through bioagents

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ABSTRACT

A field experiment was carried out at Central Research Farm, Bidhan Chandra Krishi Viswavidyalaya, Gayeshpur for two years in a row, in 2018 and 2019, to manage the rice root-knot nematode Meloidogyne graminicola using some bacterial bioagents, Bacillus pumilus, Bacillus subtilis, and Pseudomonas fluorescence by soil application in nursery beds. The chemical nematicide carbofuran was employed as a standard check. The experiment was designed in a Randomized Block Design, with five treatments reproduced six times. The experiment's results demonstrated that all treatments outperformed the untreated control in terms of seedling height, number of galls at transplanting, root knot index, soil and root nematode population at harvesting, and yield. Pseudomonas fluorescence surpassed other bacterial bioagents, yielding 1.26 t ha^{-1} in 2018 and 2.72 t ha^{-1} in 2019. However, in terms of incremental cost benefit ratio (ICBR), carbofuran treatment was the most effective, followed by bacterial bioagents.

Keywords: *Bacillus pumilus, Bacillus subtilis, Meloidogyne graminicola, Pseudomonas fluorescence* and rice

Rice is an important crop in India. Furthermore, this country has the most rice production land, as rice is one of the most important food crops. Since the country's green revolution, the number of insect and non-insect pests such as mites and nematodes has steadily increased. Farmers lose an estimated average of 37% of their rice crop to pests and diseases every year. One of the primary restrictions in rice production is the presence of many diseases and pests, the most common of which are soil-borne diseases caused by plant parasitic nematodes. Rice root-knot nematodes (*Meloidogyne* spp.), rice root nematode (*Hirschmanniella oryzae*), white tip nematode (*Aphlenchoides besseyi*), and stem nematode (*Ditylenchus angustus*) are the most common PPNs linked with rice-based cropping methods (Sharma and Rahaman, 1998). Among these, the rice root-knot nematode is a severe threat. Every 1000 nematodes present around early seedlings in highland rice reduces grain production by an estimated 2.6% (Rao and Biswas, 1973). In irrigated rice, damage occurs in nurseries before transplantation or floods in case of direct sowing. Experiments have revealed that 4000 juvenile of *M. graminicola* may drown up to 72% of deepwater rice plants (Bridge and Page, 1982). It is regarded as a major worldwide pest of rice (MacGowan and Langdon, 1989). The rice root-knot nematode, *M. graminicola*, forms terminal, hook-shaped, or spiral galls, which are distinctive of this nematode infection (Khan *et al*., 2012; Kyndt *et al*., 2013). *M. graminicola* causes significant damage to upland, rain-fed lowland, and irrigated rice farming (Prot *et al.*, 1994; Netscher and Erlan, 1993). In India, this nematode caused 16-32% production losses in rainfed and highland rice (Prasad *et al*., 2010). It is well adapted to flooded conditions and can survive in waterlogged soil as eggs in egg-masses or as juveniles for long periods. *M. graminicola* populations fall fast after four months, however some egg masses can survive for at least 14 months in wet soil. *M. graminicola* can also live in 1m deep waterlogged soil for at least 5 months. It cannot infest rice in waterlogged circumstances, but it quickly invades when contaminated soils are drained. It can live in the roots of affected plants. It prefers soil moisture of 32%.

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It thrives at moisture levels of 20-30% and soil dryness during rice tillering and panicle commencement. Its population grows alongside the development of vulnerable rice plants. Several nematode management strategies are successful for suppressing rice RKNs (Gaur and Pankaj 2010). Despite their negative effects on humans and the environment, chemical nematicides are helpful in worm management through seed treatment and soil application. Soil treatment of phorate and fosthiazate considerably lowers root gall infection in rice plants (Prasad and Rao, 1977; Rui *et al.,* 2015). However, because of poor market value of rice, chemical nematicides are not economically viable (Mantelin *et al.,* 2017). The soil characteristics and multiplication of soil-dwelling beneficial organisms are inhibited as a result of the continual use of chemicals. Furthermore, the expense of nematicides and residual concerns have made the nematode management technique unappealing to producers. Use of biocontrol agents for management of plant nematodes is currently regarded as an important component of integrated nematode management strategy. Under this context, the present experiment was carried out to assess the effectiveness of several bioagents against rice root knot nematode.

MATERIALS AND METHODS

The experiment was carried out at the Central Research Farm, Bidhan Chandra Krishi Viswavidyalaya, Nadia, during the Kharif seasons of 2018 and 2019. The experiment was set up in a complete randomised block design with five treatments (T1- Nursery bed treatment with *Bacillus pumilus* @ 20 g m2 (2×10^8 cfu g^{-1}), T2 - Nursery bed treatment with *Bacillus subtilis* @ 20 g m2 (2×10^8 cfu g^{-1}), T3 - Nursery bed treatment with *Pseudomonas fluorescence* @ 20 g m2 (2×10^8 cfu g^{-1}), T4 - Nursery treatment with carbofuran @ 1 kg a.i. ha^{-1} and T5 - Untreated check) and was replicated six times. The bioagents were supplied by the Indian Institute of Horticultural Research, Bangalore. Certified rice seeds (*Oryza sativa* L.) *M. graminicola* susceptible cv. Shatabdi were used for the experiment. The seeds were steeped in water for 12 hours before being transferred to a clean muslin bag. The bag was hung in the shade for 24 hours to promote seed germination. A seed bed was established for producing rice seedlings in nematode-infested plots with an initial nematode population of 449 and 350 per 200 cc of soil in 2018 and 2019, respectively. A separate bed was set up for each treatment, and the treatments are recommended as previously stated. After that, the seeds were sown in the nursery bed and watered regularly. On the 28th day, seedlings with four leaves and a height of 12-15 cm were transplanted onto the main field at a 20 cm × 10 cm spacing. Each plot measured 12 m^2. Each treatment was duplicated six times. The plants were collected four months after seeding, dried for two weeks, then thrashed individually using an automated thrasher to assess grain yield (with seed husks, no grain grinding). The observations were recorded on the initial nematode population (INP) per 200 cc soil (nursery and main-field), seedling height (cm), number of galls per root system before transplanting (average of 20 seedlings), root-knot index (RKI) at harvest (on 1-5 scale) (average of 20 seedlings), final nematode population (FNP) per 200 cc soil and 5 g root (main-field) at harvest, rice gain yield per plot expressed as t ha^{-1} and incremental cost benefit ratio (ICBR).

The initial population density of *M. graminicola* J2 in the nursery and main field soils, as well as the final nematode population from the main field, were estimated by collecting soil from the field and extracting nematodes from the soil using Cobb's decanting and sieving method (modified) followed by Baermann's funnel technique (Southey, 1986).

During the experiment, plants were carefully removed, and the roots were checked at the time of transplantation to count the number of galls per 20 seedlings and determine the root knot index according to 0-5 scale (Taylor and Sasser, 1978). *M. graminicola* egg masses do not form on the root surfaces and instead stay lodged in the root tissues. Thus, they were counted by tearing the galls under a stereomicroscope (Khan *et al.,* 2012). The final nematode population from 200 cc soil and 5g root in the main field was counted.

Analysis of variance (ANOVA) and least significant differences (LSDs) were used to determine treatment effects at $P\leq0.05$, 0.01 and 0.001. Data transformation using square root transformation was performed as needed.

RESULTS AND DISCUSSION

The experimental results of the year 2018 presented in table 1 revealed that all treatments gave significant good yield over untreated control, but T4, i.e. nursery treatment with carbofuran @ 1 kg a.i. ha^{-1} gave maximum rice yield (1.54 t ha^{-1}), followed by T3 (1.26 t ha^{-1}), i.e. nursery bed treatment with *P. fluorescens* @ 20 g m^{-2}. Among the other bacterial bioagents, nursery bed treatment with *B. subtilis* @ 20 g m^{-2} (T2) produced a higher yield (1.24 t ha^{-1}) than treatment with *B. pumilus* @ 20 g m^{-2} (1.15 t ha^{-1}), although these are statistically equivalent. The observation on root knot index was determined to be non-significant. Final nematode counts in 200 cc soil and 5 g root were considerably lower in all treatments compared to the untreated control.

Table 2 displayed the results for the year 2019. The results showed that all treatments produced considerably greater yields than the control. The plot treated with carbofuran, or T4, had the highest yield (3.43 t ha^{-1}), followed by T3 (nursery bed treatment with *P. fluorescens* @ 20 g m^2) (2.72 t ha^{-1}), T1 (2.24 t ha^{-1}), and T2 (2.25 t ha^{-1}). The treatments T1 and T2 were statistically equivalent in terms of yield contribution. T3 produced the highest seedling height (30 cm), followed by T2 (27.88 cm). All treatments resulted in a considerably lower number of galls at transplantation than the untreated control. The lowest gall was detected in T4, followed by T3. In terms of root knot index, final nematode population in soil, and 5 g root at harvest, all treatments outperformed the control. The lowest root knot index (1.67) was found in T4, followed by T3 (1.72). The treatment T4 resulted in the greatest percentage reduction in soil nematode (46.95%) and roots nematode (63.74%) populations at harvest, followed by T3 (34.24% and 46.20%, respectively). T1 and T2 treatments reduced soil nematode and root nematode populations at harvest by 20.17% and 24.49%, and 20.87% and 26.95%, respectively.

Table 1: Bio-management of *M. graminicola* in transplanted rice during 2018

Treatments	*Seedling height (cm)	*Number of galls per seedling at transplanting	Root Knot Index at harvest	Final Nematode Population at harvest		Yield		ICBR
				200 cc soil	5 g root	kg $plot^{-1}$	t ha^{-1}	
T_1	4.59 (20.07)	1.86 (2.48)	2.28	266.33	2867.17	1.39	1.15	0.41
T_2	4.51 (19.39)	1.75 (2.08)	2.22	257.33	2801.67	1.48	1.24	0.55
T_3	4.61 (20.28)	1.7 (1.90)	2.17	241.00	2001.00	1.51	1.26	1.58
T_4	4.51 (19.36)	1.39 (0.93)	2.11	184.00	1225.00	1.85	1.54	20.47
T_5	4.32 (17.63)	2.85 (7.11)	2.38	318.83	3922.17	1.07	0.89	-
SEm (±)	**0.05**	**0.03**	**0.15**	**21.54**	**336.82**	**0.10**	**0.09**	-
LSD (0.05)	**0.15**	**0.1**	**NS**	**63.98**	**1000.60**	**0.29**	**0.25**	-

*Note: Figure in parentheses indicate original values, * Data shown in the table are √(x+0.5) transformed values*
INP = 449 J2/200 cc soil in the nursery and 228 J2/200cc soil in the main field

Table 2: Bio-management of *M. graminicola* in transplanted rice during 2019

Treatments	*Seedling plant height (cm)	*Number of galls per seedling at transplanting	Root knot index at harvest	Final nematode population at harvest		Yield		ICBR
				200 cc soil	5 g root	kg $plot^{-1}$	t ha^{-1}	
T1	4.90 (23.04)	3.53 (11.50)	2.11	303.50	235.33	2.69	2.24	0.72
T2	5.37 (27.88)	3.58 (11.83)	1.92	300.83	227.67	2.70	2.25	0.73
T3	5.56 (30.00)	3.29 (9.83)	1.72	250.00	167.67	3.27	2.72	3.94
T4	5.29 (27.21)	3.00 (8.00)	1.67	201.67	113.00	4.12	3.43	37.11
T5	4.85 (22.58)	3.89 (14.17)	2.28	380.17	311.67	2.13	1.78	-
SEm (±)	**0.12**	**0.07**	**0.15**	**15.91**	**18.85**	**0.18**	**0.15**	-
LSD (0.05)	**0.36**	**0.21**	**0.44**	**47.26**	**55.99**	**0.54**	**0.43**	-

*Note: Figure in parentheses indicate original values, * Data shown in the table are √(x+0.5) transformed values*
INP = 350 J2 per200 cc soil in the nursery and 266 J2 per 200 cc soil in the main field

Bioagents have a role in the post-nematicide management of nematodes. The above results were in agreement with the findings of Haque, 2013 who reported that the soil application and root dip of *P. fluorescens* or *T. harzianum* + carbofuran was found most effective in increasing yield of rice and suppressed the gall formation, egg mass production and soil population of *M.*

graminicola. The application of *P. fluorescens* at 20 g m^{-2} was shown to be beneficial in lowering nematode populations and boosting grain yields. *Bacillus megaterium* significantly reduced the nematode galling (Padgham *et al.,* 2005; Anita and Samiyappan, 2012). They have reported that induction of defense enzymes phenol, peroxidase (PO), polyphenol oxidase (PPO), phenyl ammonia lyase (PAL), super oxide dismutase (SOD) and chitinase by *P. fluorescens* isolate against rice root-knot nematode resulting in significant reduction in nematode infection. *Pseudomonas* sp. and *Bacillus* sp. have been identified as important bioagents in the battle against root and soil-borne diseases in a number of crops, including wheat, tomato, potato, and chickpea (Hussain *et al*., 2020; Nguvo and Gao, 2019). Antimicrobial metabolites produced by bacteria bioagents from the genera *Agrobacterium*, *Bacillus*, *Pantoea*, *Pseudomonas*, *Serratia*, *Stenotrophomonas*, *Streptomyces*, and others have been found, with the majority of them possessing broad-spectrum activity. *Bacillus* has been researched for lipopeptides such as iturin, surfactin, and fengycin, whereas *Pseudomonas* has been examined for antibiotic metabolites such as DAPG, pyrrolnitrin, and phenazine (Kenawy *et al.,* 2019; Dimkic *et al*., 2022). To minimize soil-borne infections, plant growth released toxic surface chemicals (biosurfactants) and volatiles, chitinase cell wall-degrading enzymes, and 1, 3-glucanase-enhancing rhizobacteria (Youssef *et al.,* 2018; Wang *et al.,* 2021). The release of siderophore ligands, which efficiently capture iron and impede pathogen growth, was previously discovered as a biocontrol mechanism (Parray *et al*., 2019; Avelar, 2021).

CONCLUSION

Food security is a primary issue for everyone across the world, but it is especially critical in developing countries like India, which have fast rising populations. Food crop output must increase to meet the nutritional needs of a rising population. Overuse of synthetic pesticides, is an unsustainable practice in terms of both environmental and organism consequences. In this context, it can be concluded from the experiment that among the six treatments including check and untreated, although the minimum number of nematode population were observed in check i.e. nursery treatment with carbofuran @ 1 kg *a.i.* ha^{-1} with maximum grain yield, the next best treatment was nursery bed treatment with *P. fluorescence* @ 20 g m^{-2} with respect to other bacterial bioagents.

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