Journal of Entomology and Zoology Studies

Available online at www.entomoljournal.com

Rudra Pratap Subudhi
M.Sc. (Ag.), Department of
Nematology, College of
Agriculture, Odisha University
of Agriculture and Technology,
Bhubaneswar, Odisha, India

Niranjan Das
Assistant Professor, Department
of Nematology, College of
Agriculture, Odisha University
of Agriculture and Technology,
Bhubaneswar, Odisha, India

Tamireddy Anjali
Ph.D. Research Scholar,
Department of Nematology,
College of Agriculture, Odisha
University of Agriculture and
Technology, Bhubaneswar,
Odisha, India

Amit Ahuja
Ph.D. Research Scholar,
Division of Nematology, ICAR-
Indian Agricultural Research
Institute, New Delhi, India

Sachin Gangwar
Ph.D. Research Scholar,
Division of Nematology, ICAR-
Indian Agricultural Research
Institute, New Delhi, India

Effect of bacterial antagonists on multiplication of root-knot nematode (*Meloidogyne graminicola*) in rice

Rudra Pratap Subudhi, Niranjan Das, Tamireddy Anjali, Amit Ahuja and Sachin Gangwar

Abstract
Rice (*Oryza sativa* L.) is the most consumed cereal in the world. Rice production faces a lot of abiotic and biotic challenges. Among the biotic factors rice Root-knot nematode, *Meloidogyne graminicola* parasitism results in substantial yield loss. Their management by using bio-control agents is a recent trend now-a-days. A pot culture experiment was carried out in the net house condition in the Department of Nematology, College of Agriculture, O.U.A.T, Bhubaneswar, Odisha during 2018-19 to assess the effect of the bacterial antagonists viz. *Bacillus pumilus, Bacillus subtilis, Pseudomonas fluorescens* on multiplication of root-knot nematode. The results revealed that all the treatments were superior over the untreated check concerning the reduction in the multiplication of root-knot nematodes. Soil application of Carbofuran @ 60mg/pot with 1 kg soil at the time of sowing exhibited maximum decrease of root-knot nematode population over untreated check followed by soil application of *Pseudomonas fluorescens* @ 20mg/pot with 1 kg soil by registering decreased number of galls, number of egg masses, nematode population in 200cc of soil and total nematode population in soil and root by 79.33%, 83.14%, 64.29%, and 67.09% respectively.

Keywords: Rice, *Meloidogyne graminicola*, bacillus, pseudomonas

Introduction
Rice is infected by more than two hundred species of parasitic nematode [1]. Several ecto and endoparasites of root, stem and foliar parts have been reported in the rice crop from the rice-wheat cropping system of the Indo-Gangetic plains, causing damage to the tune of 10.54 percent in rice alone [2]. Rice crop is attacked by *Meloidogyne incognita, M. graminicola, M. javanica, M. oryzae* and *M. arenaria*. They are cosmopolitan, obligate endo-parasite and examples of highly adopted root parasites affecting rice plants in all rice ecosystems in various countries across the globe. Amongst these species, *M. graminicola* being a predominant pest of rice is a threat to rice cultivation in Southeast Asia, which contribute maximum in world's total rice production and consumption. In the view of high yield losses caused by rice root-knot nematodes, it is necessary to minimize crop damage by adopting environment-friendly management methods. Among all the possible safe alternatives to pesticides for disease management biological control is one of them as it is likely to be free from toxic residual effects. There are many microbial bio-agents of root-knot nematodes and their application can cause satisfying decrease in the nematode number. *Pseudomonas fluorescens* and *Bacillus* spp. are among the most commonly used bacterial bio-control agents (BCAs) against plant-parasitic nematodes. However, different bio-agent species vary in their ability to reduce population levels of specific nematode pests and the use of such a method is also influenced by other factors such as the target nematode pest species, soil temperature, and others. With this background three bio-agents viz. *Bacillus pumilus*, *Bacillus subtilis,* and *Pseudomonas fluorescens* were compared along with a standard nematicide Carbofuran and untreated control to study the effect of bacterial antagonists on multiplication of root-knot nematode in rice.

Materials and Methods
The experiment was carried out in pot culture condition during *Kharif,* 2018 following Completely Randomized Design (CRD) in the net house of Department of Nematology, College of Agriculture, O.U.A.T, Bhubaneswar.

Corresponding Author:
Rudra Pratap Subudhi
M.Sc. (Ag.), Department of
Nematology, College of
Agriculture, Odisha University
of Agriculture and Technology,
Bhubaneswar, Odisha, India

For nematode infection, well pulverized sandy loam infected soil was collected from a sick plot of nematodes. Spreading of the collected soil was done on a transparent polythene sheet before mixing the, thoroughly. 3 composite soil samples of 200cc each were collected for screening and estimation of the initial nematode population. Based on the shifting and gravitation principle processing of 200cc soil was done by Cobb's sieving [3] and modified Baermann's funnel technique [4]. The nematodes collected from different mesh sieves were compounded and poured over a moist double-layered tissue paper supported on an aluminium wire gauge. When the water got drained the tissue paper assembly was kept over a Petri dish filled with water touching the bottom of aluminium wire gauge. The whole system was kept undisturbed for 24 hrs for movement of the nematode through the tissue paper into the water in the Petri dish. The nematode suspension so obtained was examined under a binocular stereoscopic microscope for preliminary observation. The nematodes in the suspension were killed by immersing the bottle in boiling water for about 3-4 min with constant stirring. An equal volume of double strength formalin (8% v/v) was added to reduce the final strength to 4%. Then the suspension was taken in a 7×7 square counting dish, the nematode species were identified and their numbers were counted under a stereoscopic microscope. Then cleaning and surface sterilization of earthen pots of 15 cm diameter was done in 1% formaldehyde solution before making them air dry. Pots were then filled with naturally infested soil (1 kg) collected from the sick plot. Bio-agents were added to the potting soil as per the treatment requirements given below. Different treatments under observation were fixed on basis of the presence of various

bacterial antagonists such as T1 - *Bacillus pumilus* @20mg/pot (1 kg soil), T2 - *Bacillus subtilis* @20mg/pot (1 kg soil), T3 - *Pseudomonas fluorescens* @ 20mg/pot (1 kg soil) along with Carbofuran @ 60mg/pot (1 kg soil) as T_4 and an untreated check as T_5. For the experiment seeds of one susceptible variety of rice *i.e.*, Naveen was surface sterilized in 2.5% Sodium hypochlorite solution for two minutes followed by rinsing thrice with distilled water and air-dried in shade. Three to four seeds were sown in each pot, lightly covered with soil and sprinkled with water to keep the soil moist. After germination of the seeds, thinning was done keeping one plant per pot. Weeding and watering were done as per requirement. Forty-five days after sowing the number of galls, the number of egg masses, the final nematode population in soil recorded during the investigation was subjected to statistical analysis in a complete randomized design. Fisher's methods of analysis of variance at a 5% level of significance were followed. Further, the comparison of the treatment means was done by calculating standard error of mean and least significant difference were recorded.

Results

The following three species of plant-parasitic nematodes along with some saprophytes were detected from the soils collected from the sick plot of AICRP on Nematodes with prior cultivation of rice for experimentation. Among these plant-parasitic nematodes, only the root-knot nematode (*Meloidogyne graminicola*) population was found to be above pathogenic level (216.33 J_2 /200 cc soils) and the rest others were below pathogenic level (29.23 – 55.26 J_2 /200cc soil).

Fig 1: Plant Growth 25 Days after Sowing In Infected Soil

Table 1: Nematode population of soil collected for experimentation

Sl no	Nematode name	Population	Pathogenicity
1	*Meloidogyne graminicola*	216.33	Above pathogenic
2	*Hirschmaniella oryzae*	55.26	Below pathogenic
3	*Pratylenchus indicus*	29.23	Below pathogenic
4	*Saprophytes (Dorylaimids & Rhabditids)*	78.64	_____

Effect of bio-agents and Carbofuran on Initial Soil Nematode Population

Table 2. Indicated that the application of bio-agents and Carbofuran reduced the initial population of nematode of the experimental pot soil. The reduction percentage was in the range of 50.30% - 64.29%. There was no significant difference between the effect of *Bacillus pumilus* (T1) and *Bacillus subtilis* (T2) in 20 respect of the reduction of the root-knot nematode population. However, *Pseudomonas fluorescens* (T3) was among bio-agents was comparatively better in the reduction of the population of root-knot nematode. It reduced the population by 55.27%. Among all

the treatments the Carbofuran (T4) was distinctly superior over others in respect of population reduction of root-knot nematode (*Meloidogyne graminicola*). The initial population was reduced by 64.29% by this treatment. The population of *Hirschmaniella oryzae* was reduced in all the treatments except control (T5). The reduction percentage was in the range of 10.42% to 29.87%. Carbofuran again was found to reduce the *Hirschmaniella oryzae* population maximum by 29.87% followed by the application of *Pseudomonas fluorescens* (T3) 18.56%. Similarly, the population of *Pratylenchus indicus* was reduced in all treatments except control. Maximum reduction was recorded by Carbofuran (20.45%) followed by *Bacillus pumilus* (14.47%). *Pseudomonas fluorescens* recorded an 11.05% reduction of *Pratylenchus* population. On the other hand the population of saprophytes enhanced in T1 (*Bacillus pumilus*), T2 (*Bacillus subtilis*) and T5 (Untreated check). It was also maintained in T3 (*Pseudomonas fluorescens*) with only a 2.4% reduction. But in T4 Carbofuran reduced the saprophyte population by 23.7%.

Fig 1: Root growth and galls produced in plants of different treatment

Table 2: Effect of bio-agents and Carbofuran on Soil Nematode Population (Average of 4 replications)

Treatment	*Meloidogyne graminicola*			*Hirschmaniella oryzae*			*Pratylenchus indicus*			Saprophytes		
	INP	FNP	% Change over INP	INP	FNP	% Change over INP	INP	FNP	% Change over INP	INP	FNP	% Change over INP
T$_1$		107.50	(-) 50.30		44.75	(-)19.01		25.0	(-)14.47		83.75	(+)6.49
T$_2$		102.50	(-) 52.61		49.50	(-)10.42		26.75	(-)8.48		80.25	(+)2.04
T$_3$	216.33	96.75	(-) 55.27	55.26	45.00	(-)18.56	29.23	26.00	(-)11.05	78.64	76.75	(-)2.40
T$_4$		77.25	(-) 64.29		38.75	(-)29.87		23.25	(-)20.45		60.00	(-)23.70
T$_5$		230.25	(+) 6.04		61.75	(+)11.74		35.50	(+)21.45		94.00	(+)19.53
SEm(±)	-------	1.17	-------	-------	0.98	-------	-------	0.94	-------	-------	1.02	-------
CD(0.05)	-------	3.53	-------	-------	2.94	-------	-------	2.82	-------	-------	3.07	-------

T$_1$ = Sowing of seeds in infested soil enriching with *Bacillus pumilus* @20mg/pot (kg soil)

T$_2$ = Sowing of seeds in infested soil enriching with *Bacillus subtilis* @20mg/ pot (kg soil)

T$_3$ = Sowing of seeds in infested soil enriching with *Pseudomonas fluorescens* @20mg/ pot (kg soil)

T$_4$ = Sowing of seeds in infested soil with application of Carbofuran @60mg/ pot (kg soil)

T$_5$ = Untreated check

INP - Initial Nematode population/200 cc soil

FNP - Final Nematode population/200 cc soil

Table 3: Effect of bio-agents and Carbofuran on root-knot nematode multiplication (Average of 4 replications)

	No. of galls/root system	% decrease over control	No. of egg masses /root system	% decrease over control	INP/ 200cc soil	FNP/ 200cc Soil	NP In Root	Total NP	Rf = Pf/Pi
T$_1$	12.0	68.00	7.75	65.16		107.50	15.75	553.25	0.5
T$_2$	14.0	62.00	7.50	66.29		102.50	17.5	530	0.4
T$_3$	10.50	72.00	5.0	77.52	216.33	96.75	12.25	496	0.4
T$_4$	7.75	79.33	3.75	83.14		77.25	8	394.25	0.3
T$_5$	37.50	---------	22.25	---------		230.25	46.75	1198	1.1
SEm(±)	0.94	---------	1.81	---------	-------	1.17	0.60	-------	-------
CD(0.05)	2.82	---------	2.44	---------	-------	3.53	1.79	-------	-------

T$_1$ = Sowing of seeds in infested soil enriching with *Bacillus pumilus* @20mg/pot (kg soil)

T$_2$ = Sowing of seeds in infested soil enriching with *Bacillus subtilis* @20mg/pot (kg soil)

T$_3$ = Sowing of seeds in infested soil enriching with *Pseudomonas fluorescens* @20mg/pot (kg soil)

T$_4$ = Sowing of seeds in infested soil enriching with Carbofuran @60mg/pot (kg soil)

T$_5$ = Untreated check

 INP - Initial Nematode population/200 cc soil

FNP - Final Nematode population/200 cc soil

NP - Nematode Population

Rf - Reproductive factor

Effect of bio-agents and Carbofuran on root-knot nematode multiplication

According to Table 3, the number of galls/root systems was found to be minimum in T4 (Carbofuran). Among the bacterial antagonists, *Pseudomonas fluorescens* (T3) exhibited the lowest number of galls/root system (10.50). It was 72% less than control. The final root-knot nematode population both in the soil as well as plant root was found to be influenced by all the treatments including untreated control. It was a minimum (77.25 J2/200 cc soil & 8.00/root system) in Carbofuran treated plot followed by 96.75 J2/200 cc soil and 12.25/root system in T3 (*Pseudomonas fluorescens*). The reproduction rate was minimum in T4 (0.3) and maximum in T5 (1.1). Among bio-agents, T2 (*Bacillus subtilis*) and T3 (*Pseudomonas fluorescens*) were found to have an equal effect on the multiplication of root-knot nematode with reproduction rate 0.4 each while T1 (*Bacillus pumilus*) reduced the multiplication with reproduction rate 0.5.

Discussion

From the experimental result, it was found that there was a substantial decrease in the number of root galls, egg masses and nematode populations in roots and soil, in all the treatments over the untreated check. Statistical analysis of data cited in Table 3 revealed that percentage of decrease in the number of galls per plant root (79.33%), number of egg masses per plant root (83.14%) over untreated check was seen in the treatment T4 (Carbofuran@ 60mg/pot (kg soil). This is due to the toxic effect of nematicide Carbofuran on the nematodes inside the soil which showed decreased hatching of eggs and movement of larvae into roots, as the consequences their effect on plant growth was less. The population of *M. graminicola* and percentage of plant infections decreases if Carbofuran 3G is used in pre-plant application [5]. Thus Carbofuran application in paddy fields controls *M. graminicola* effectively [6]. The next best treatment with this respect was *Pseudomonas fluorescens* which has the almost same impact as nematicide check without the disadvantages possessed by the chemical Carbofuran. The effectiveness of *Pseudomonas fluorescens* in reducing nematode population may be attributed due to its inherent property which may benefit plant growth by producing toxic metabolites inhibiting nematodes and eliminating microorganisms [7]. Reduction in nematode population in soil treated with *Bacillus subtilis, Bacillus pumilus* were at par. Genus *Bacillus* is a large group of bacteria that affects the plant-parasitic nematodes adversely. *Bacillus subtilis* is one of the *Bacillus spp* with nematicidal effects [8] and numerous *Bacillus* strains have been found to express activities that suppress nematodes [9].

Conclusion

Among all treatments, Carbofuran@ 60mg/pot (1 kg soil) reduced the maximum population of root-knot nematode followed by *Pseudomonas fluorescens* @20mg/pot (1 kg soil). Ultimately, this research information may be treated as a versatile and valuable tool to formulate the field trials for further in-depth study.

References

1. Prot JC. Effects of economic and policy changes on status of rice nematode pests in Vietnam and the Philippines, Fundamental and Applied Nematology. 1994; 17:195-198.
2. Jain RK, Mathur KN, Singh RV. Estimation of losses due to plant parasitic nematodes on different crops in India, Indian Journal of Nematology. 2007; 37:217-219.
3. Cobb NA. Estimating the nematode population of the soil; Agric. Teeh. Cire. Bur. PI. Ind. U.S. Dep. Agrie., 1918; 1:48.
4. Schindler AF. A simple substitute for a Baermann funnel. Plant Dis. Rept., 1961; 45:747-748.
5. Rahman ML. Evaluation of nematicides to control root-knot nematode (*Meloidogyne graminicola*) in deep water rice. Current Nematology. 1991; 2:93-98.
6. Soriano IR, Reversat G. Management of *Meloidogyne graminicola* and yield of upland rice in South-Luzon, Philippines, Nematology. 2003; 5:879-884.
7. Anitha B, Rajendran G, Samiyappan R. Induction of systemic resistance in tomato against root-knot nematode, *Meloidogyne incognita* by *Pseudomonas fluorescens*, Nematologia Mediterranea. 2004, 32(1).
8. Gokte N, Swarup G. On the potential of some bacterial biocides against root-knot and cyst nematodes, Indian. Journal of Nematology. 1988; 18:78-83.
9. Siddiqui ZA, Mahmood I. Role of bacteria in the management of plant parasitic nematodes: A review Bioresource Technol. 1999; 69:167-179.