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The Pharma Innovation

ISSN (E): 2277- 7695
ISSN (P): 2349-8242
NAAS Rating: 5.03
TPI 2019; 8(7): 412-414

www.thepharmajournal.com
Received: 13-05-2019
Accepted: 15-06-2019

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Effect of *Bacillus pumilus*, *Bacillus subtilis* and *Pseudomonas fluorescens* on plant growth parameters of rice infected by root-knot nematode (*Meloidogyne graminicola*)

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Abstract
Rice (*Oryza sativa* L.) is the most important food crop of the developing world and the staple food of more than half of the world's population. Its production and productivity is influenced by so many biotic and abiotic factors. Plant parasitic nematodes are considered as one of the major causes among them. Root-knot nematode, *Meloidogyne graminicola* is the predominant species causing substantial yield loss mostly in upland and medium land situation. Management of root-knot nematode (*Meloidogyne graminicola)* through use of bio-control agents is a recent trend. A pot culture experiment was thus carried out in net house condition in the Department of Nematology, College of Agriculture, O.U.A.T, Bhubaneswar, during 2018-19 to assess the effect of the bacteria bio-agents viz. *Bacillus pumilus, Bacillus subtilis, Pseudomonas fluorescens* on plant growth. The experiment revealed that all the treatments were superior over untreated check with respect to growth parameters of rice plant. Soil application of Carbofuran @ 60mg/pot with 1 kg soil at the time of sowing exhibited maximum increase in growth parameters of rice plant over untreated check followed by soil application of *Pseudomonas fluorescens* @ 20mg/pot with 1 kg soil registering increased shoot length, root length, fresh shoot weight, dry shoot weight, fresh root weight and dry root weight by 16.08%, 32.72%, 50.26%, 59.49%, 48.00% and 44.28% respectively.

Keywords: Rice, nematode, *Bacillus*, *Pseudomonas*

Introduction
Rice (*Oryza sativa* L.) is one of the most important staple food crops of India which is a major source of calories for about 60 per cent of world population. India is the second largest rice producer in the world following China (209.5 million tonnes) producing 110.15 million tonnes in 43.19 million ha area with the yield rate 2550 kg/ha. In Odisha, rice is cultivated in an area of 3.88 million ha with production of 8.38 million tonnes and average productivity of 2162 kg/ha. (Agricultural Statistics 2017). Over 200 species of plant parasitic nematodes have been reported to be associated with rice (Prot, 1994) [4]. A number of 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 per cent in rice alone (Jain *et al*., 2007) [3]. Rice crop is attacked by *M. incognita*, *M. graminicola*, *M.* triticoryzae, *M. javanica*, *M. oryzae* and *M. arenaria*. They are cosmopolitan, affecting rice plants in all rice ecosystems in various countries across the globe. Amongst these species, *M. graminicola* is a primary pest of rice and poses a substantial threat to rice cultivation in particular Southeast Asia, where, around 90% of the world rice is grown and consumed. Rice root-knot nematode, *M. graminicola* has emerged as a pest of international importance. In view of the enormity of the yield losses caused by rice root-knot nematodes, it is necessary to minimize crop damage by adopting environment friendly management methods. Biological control is one possible safe alternative to pesticides for disease management, and is likely to be free from toxic residual effects. There are numerous microbial antagonists of root-knot nematodes and their application results in significant decrease in the nematode populations. *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 growth parameters of root-knot nematode infected rice.

Materials and methods

The experiment was carried out in pot culture condition during *Kharif*, 2018 following Completely Randomized Design (CRD) in net house of Department of Nematology, College of Agriculture, O.U.A.T, Bhubaneswar. For nematode infection, well pulverised sandy loam infected soil were collected from sick plot of nematodes. The soil was spread on a clean polythene sheet, mixed thoroughly and three composite samples amounting to 200cc each were drawn for screening to estimate the initial nematode population. 200cc of soil was taken and processed by Cobb's sieving (Cobb, 1918) [2] and modified Baermann's funnel technique (Schindler, 1961) [7] based on the principles of shifting and gravitation. 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 petridish 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 petridish. The nematode suspension so obtained was examined under a binocular stereoscopic microscope for preliminary observation. The nematodes in the suspension were killed by emerging 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. Earthen pots of 15 cm diameter were cleaned and surface sterilized in 1% formaldehyde solution and made air dry. Pots were then filled with naturally infested soil (1 kg) collected from sick plot. Bio-agents were added to the pot soil as per the treatment requirements given below. Different treatments under observation were fixed on basis of presence of various bacterial antagonists such as T_1 - *Bacillus pumilus* @20mg/pot (1 kg soil), T_2 - *Bacillus subtilis* @20mg/pot (1 kg soil), T_3 - *Pseudomonas fluorescens @ 2*0mg/pot (1 kg soil) along with chemical nematicide *i.e.* Carbofuran @ 60mg/pot (1 kg soil) and untreated check. For the experiment seeds of one susceptible variety of rice *i.e.* Naveen were 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 observations on different plant growth parameters such as shoot length, root length, fresh and dry shoot weight, fresh and dry root weight various observation recorded during the course of investigation were subjected to statistical analysis in a complete randomized design. Fisher's methods of analysis of variance at 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 shoot length of rice plant exhibited distinct variation in the treatments. The percentage change over control was in the range of 1.69% to 16.08%. Among all the treatments, T_4 (application of Carbofuran @60mg/pot (1 kg soil) exhibited highest shoot length (42.37cm) followed by T_3 (41.32 cm) *i.e.* (soil enriching with *Pseudomonas fluorescens* @20mg/pot (1 kg soil) and they are statistically at par but significantly different over T_1 and T_2. All four treatments enhanced the root length. The plants in all the treatments exhibited enhanced root length as compared to untreated check .The root length was in the range of 13.37cm to 17.32cm. Maximum enhancement of root length was observed in T_4 (32.72%) with application of Carbofuran@ 60mg/pot (1 kg soil) followed by T_3 (21.91%) i.e. *Pseudomonas fluorescens* @ 20mg/pot (1 kg soil). The treatments T_1 (*Bacillus pumilus* @20mg/pot (1 kg soil) & T_2 (*Bacillus subtilis* @20mg/pot (1 kg soil)*)* were significantly inferior from other treatments with variations ranging from 2.45% to 11.11% respectively. The fresh shoot weight in different treatments was in the range of 7.07g to 8.52g. All bio-agent treatments exhibited positive result for fresh shoot weight along with Carbofuran treatment. T_1 & T_2 were at par but significantly inferior than T_4 which exhibited the highest fresh shoot weight. The dry shoot weight in different treatments was in the range of 2.20g to 2.77g. Maximum change of dry shoot weight over control was 59.49% in case of T_4 followed by 39.54% in case of T_3 and T_1 (24.29%) being the lowest.. The fresh root weight varied between 3.45 g to 4.07g. Maximum root weight (4.07g) was observed in T_4 followed by T_3 (3.70g). Among the four treatments lowest fresh root weight was recorded in T_2 (3.40g) which was at par with T_1 (3.45g). The change in dry root weight was observed in all most all the treatments. Maximum being in T_4 (2.02g) with application Carbofuran@ 60mg/pot (1 kg soil) followed by T_3 (1.80g) i.e. application of *Pseudomonas fluorescens* @20mg/pot (1 kg soil). However, the other treatments i.e. T_1 & T_2 in respect of dry root weight were at par weighing 1.62g and 1.67g respectively.

Table 1: Effect of bio-agents and Carbofuran on plant growth parameters

Treatments	Shoot Length (cm)		Root Length (cm)		Fresh shoot weight (g)		Fresh root weight (g)		Dry shoot weight (g)		Dry root weight (g)	
	Mean	% increase over control	Mean	% increase over control	Mean	% increase over control	Mean	% increase over control	Mean	% increase over control	Mean	% increase over control
T_1	37.12	1.69	13.37	2.45	7.07	24.69	3.45	25.45	2.20	24.29	1.62	15.71
T_2	38.37	5.12	14.50	11.11	7.15	26.10	3.40	23.63	2.25	27.11	1.67	19.28
T_3	41.32	13.20	15.91	21.91	7.50	32.27	3.70	34.54	2.47	39.54	1.80	28.57
T_4	42.37	16.08	17.32	32.72	8.52	50.26	4.07	48.00	2.77	59.49	2.02	44.28
T_5	36.50	-------	13.05	-------	5.67	-------	2.75	-------	1.77	-------	1.40	--------
SEm(±)	0.67	-------	0.43	-------	0.17	-------	0.08	-------	0.08	-------	0.05	-------
CD(0.05)	2.01	-------	1.29	-------	0.50	-------	0.23	-------	0.26	-------	0.14	-------

T_1 = Sowing of seeds in infested soil enriching with *Bacillus pumilus* @20mg/pot (1 kg soil)
T_2 = Sowing of seeds in infested soil enriching with *Bacillus subtilis* @20mg/pot (1 kg soil)
T_3 = Sowing of seeds in infested soil enriching with *Pseudomonas fluorescens* @20mg/pot (1 kg soil)
T_4 = Sowing of seeds in infested soil with application of Carbofuran @60mg/pot (1 kg soil)
T_5 = Untreated check

Discussion

From the experimental result, it was found that there was a substantial increase of plant growth parameters like shoot length, fresh & dry shoot weight, root length, fresh & dry root weight in all the treatments over untreated check. Statistical analysis of data cited in Table 1 revealed that highest percentage of increase in shoot length (16.08%), root length (32.72%), fresh shoot weight (50.26%), dry shoot weight (59.49%), fresh root weight (48.00%), dry root weight (44.28%) over untreated check was seen in the treatment T_4 - Carbofuran@ 60mg/pot (1 kg soil). This might be due to the quick knock down effect of chemical nematicide Carbofuran on the nematodes in soil thereby preventing or limiting the hatching of eggs and the movement of larvae into roots resulting in less effect on plant growth. This is in agreement with the findings of Rahman (1991) [5] who confirmed that Carbofuran 3G as pre plant treatment reduced the population of *M. graminicola* in the soil and the percentage of plant infestation. Similar results have earlier been reported by Soriano and Reversat (2003) [6] who stated that application of Carbofuran in rice fields effectively controlled *M. graminicola*. Another finding of Vinod and Jain (2010) [8] confirmed that shoot length, shoot dry weight were highest in chemical treatments as compared to bio-agents. The next best treatment with this respect was *Pseudomonas fluorescens* which has almost same impact as nematicide check without the disadvantages possessed by the chemical Carbofuran. The effectiveness of *Pseudomonas fluorescens* in enhancing plant growth (shoot and root) may be attributed due to its inherent property which may benefit plant growth by providing growth regulators or by producing toxic metabolites inhibiting nematodes and excluding other deleterious microorganisms. These findings are in accordance with the results cited by Anitha *et al*. (2004) [1].

Conclusion

Among all treatments T_4 - Carbofuran@ 60mg/pot (1 kg soil) was found to be significantly effective treatment in terms of enhancement of all plant growth parameters 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.

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