RESEARCH ARTICLE

EFFICACY OF BIO-AGENTS AND CARBOFURAN AGAINST ROOT KNOT NEMATODE, *MELOIDOGYNE GRAMINICOLA* INFESTING RICE

Sachin Kumar Jain[1]*, Kamal Khilari[1], Ramji Singh[1], Prashant Mishra[1], Gaje Singh[2], M. K. Yadav[3]

[1]Department of Plant Pathology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Modipuram, Meerut -250 110
[2]Department of Entomology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Modipuram, Meerut -250 110
[3]Department of Ag. Biotechnology, Sardar Vallabhbhai Patel University of Agriculture and Technology, Modipuram, Meerut -250 110
Email: sachinjain1115@gmail.com

Received-09.07.2016, Revised-25.07.2016

Abstract: Rice root-knot nematode (*Meloidogyne graminicola*) is an emerging problem to rice cultivation in various rice growing areas. Rice root knot nematode has reported to cause upto 50% loss in grain yield and in severe cases it may go upto 64 per cent. An experiment was conducted to study the efficacy of bio-agents against *Meloidogyne graminicola* on rice variety PB-1121. It was observed that, all *Trichoderma* isolates at 20 and 30 gm/kg soil significantly increased the shoot length of rice plants. *Trichoderma* isolates were reduced the infestation of root knot nematode in rice. At 40 days after seed sowing, minimum 1.61 galls/ plant were recorded in *Trichoderma* isolate-V @ 30 gm/kg soil. In case of *Trichoderma* isolate-III @ 30 gm/kg soil, average 3.00 galls/ plant were recorded as compare to carbofuran @ 3gm/kg (4.61) and control (25.17). *In vitro*, at 72 hours after inoculation, maximum 96.68% larval mortality was recorded in *Pseudomonas fluorescens* suspension. In *Trichoderma* isolate-IV, 74.18% larval mortality was recorded as compare to carbofuran (95.00%).

Keywords: Rice crop, Rice root knot nematode, *Meloidogyne graminicola*, Management

INTRODUCTION

Rice is an important cereal crop and growing all over the world. Rice grows in India is primarily divided into Basmati rice and non-Basmati rice. India and Pakistan is the major producer and suppliers of basmati rice to the world consumers. Many biotic and abiotics stresses are responsible of low production of rice. In biotics stresses, many fungi, bacterial, virus and nematode are responsible to cause serious diseases in rice. Root knot nematodes, *Meloidogyne graminicola* is an important pests attacking rice in all rice growing countries. Rice root knot nematode causes significant yield losses of rice production in upland and rainfed lowland (Jairajpuri and Baqri, 1992 and Soriano *et al*., 2000). The use of rice seedlings from non-treated nursery beds has result heavy yield loss of rice grain of 38% in comparison to 29% when rice seedlings from treated nursery beds were used (Gaur, 2003). Crop losses to the extended up to 60-100% has been reported by Dabur and Jain, (2005). Nationally rice root knot nematode, *M. graminicola* is reported to cause upto 50% loss in grain yield (Rao and Biswas, 1973). Losses in grain yield were also estimated to range from 16-32 % due to this nematode (Rao and Biswas, 1973). Carbofuran has been recommended to control nematodes in various crops (Vyas and Patel, 2001). Using of this chemical pollutes to environment and cause hazard in animal and human being. So public want some alternative control measures which safe in formulation and don't pollute to the environment. Other than chemical agents, various bio-agents fungi and bacteria are using in integrated pest management (Holland *et al*., 1999; Meyer *et al*., 2004) to control plant parasitic nematodes. Among the antagonistic fungi, *Trichoderma* species have been used as biocontrol agents against nematodes. Soil treatment by *T. harzianum* and *T. koningii* resulted in a reduction in egg production of *M. arenaria* (Rao *et al*., 1998; Sharon *et al*., 2001). Therefore, an experiment was conducted to study the comparative effect of bio-agents and carbofuran against rice root knot nematode.

*Corresponding Author

MATERIAL AND METHOD

The experiment was conducted in earthen pots in year 2014 and 2015. Earthen pots were filled by previous year maintained nematode sick soil. The presence of nematodes in sick soil was observed before fill the sick soil in pots.

For test the efficacy of *Trichodrema* isolates and *Pseudomonas fluorescens* against rice root knot nematode, five *Trichoderma* isolates and one *Pseudomonas fluorescens* isolate were obtained from the Nematology Laboratory, Department of Plant Pathology, S.V.P. University of Agriculture & Technology, Meerut, (U.P.). Before the used of bio-agents (*Trichoderma* isolates and *Pseudomonas fluorescens*), collected isolates were grown on different substrates. Wheat grains were used for mass culture of *Trichoderma* isolates. Wheat grains were soaked in water for 12 hours and then spread on

paper to remove the extra water. Dextrose was added in wheat seeds @ 20 gm/kg seed and then 250 gm of wheat grain were taken in each 500 ml conical flasks. Flasks with wheat grains were plugged with nonabsorbent cotton and wrapped with aluminium foil and then sterilized in autoclave at 121^0C temperature at 15 lbs pressure/inch^2 for 15 minutes. The sterilized wheat grains were inoculated with 5 mm diameter PDA discs punched from the periphery of actively growing 5 days old culture of *Trichoderma* isolates. All inoculated conical flasks were incubated in a BOD incubator at 26±2 °C temperature. *Trichoderma* isolates were allowed to grow with periodic shaking of the flasks, so that the surface of all wheat seeds colonized with growth of *Trichoderma* properly. For preparation of mass culture of *Pseudomonas fluorescens*, one liter capacity conical flasks containing 500 ml King's 'B' (Broth) were autoclaved at 121^0C temperature at 15 lbs pressure/inch^2 for 15 minutes. After cooling of the medium, each flask was inoculated with 1.0 ml of *P. fluorescens* culture. The flasks were kept at 26±2 °C temperature in the BOD incubator for 5 days and were shaken twice a day.

All *Trichoderma* isolates were used @ 10, 20 and 30 gm/kg soil and *Pseudomonas fluorescens* was used @ 10 ml/kg soil. One chemical (carbofuran) was also used @ 3 gm/kg soil as check the comparative effect of treatments. Three replications were maintained of each treatment. After two days of mixing of each bio-agent and chemical in pot soil, ten seeds of rice (PB-1121) were sown in each pot. At the time of observation, rice plants were carefully uprooted by lifting the roots and washed free of soil particles under slow running water. Observations were recorded on the growth of plants with respect to shoot height (cm), fresh shoot weight (g), dry shoot weight (g), root length (cm), fresh root weight (g), dry root weight (g) and development of galls per plant at 40 days after seed sowing.

In vitro effect of filtrate of bio-agents was tested against J_2 of *M. graminicola*. For obtaining the filtrate of bio-agents, *Trichoderma* isolates were first cultured on Potato dextrose broth medium and *Pseudomonas fluorescens* was on King 'B' broth medium. 5 mm bits of PDA with *Trichoderma* mycelia and 1 ml suspension of *Pseudomonas fluorescens* were added to flasks containing 200 ml of Potato dextrose broth (PDB) and King 'B' broth medium respectively. The flasks were shaken regularly. After one week, culture was filtrate through What man filter paper No.1. The suspension was then centrifuged at 5000 rpm for 20 min at 20°C to remove small sections and spores. Sterile water was used as the absolute control. For each treatment, 40 J2 of *M. graminicola* were added in Petri plates containing filtrate solution and sterile water. Mortality of the J_2 of *M. graminicola* was recorded at 24, 48 and 72 hours after inoculation.

RESULT AND DISCUSSION

Effect of bio-agents on galls/plant

Data present in **Table-1** are the mean of both years 2014 and 2015. Data of galls/plant indicates that, minimum galls (1.61 gm) were recorded in *Trichoderma* isolate-V @ 30 gm/kg soil which was differed significantly than other treatments. In case of *Trichoderma* isolate-III average 3.00 galls/ plant were recorded which was statistically at par with *Trichoderma* isolate-II (3.06) and *Trichoderma* isolate-I (3.50) when applied as 30 gm/kg soil. Average 4.61 galls/ plant were recorded in carbofuran @ 3gm/kg which was at par of *Trichoderma* isolate-I @ 10 gm/kg soil (5.62) and *Trichoderma* isolate-I @ 20 gm/kg soil (5.89). In *Trichoderma* isolate-V average 6.22 galls/ plant were recorded followed by *Trichoderma* isolate-IV (6.28) when applied as 20 gm/kg soil and *Trichoderma* isolate-V @ 10 gm/kg soil (6.34). In case of *Pseudomonas fluorescens* @ 10 ml/kg soil average 8.00 galls/ plant was recorded. All *Trichoderma* isolates, *Pseudomonas fluorescens* and carbofuran were statistically significant as compared to control (25.17).

Effect of bio-agents on plant growth parameters:

Data present in Table-1 indicates that shoot and root length of rice plant were increased in pots which were inoculated with bio-agents compare to control in both years 2014 and 2015. Maximum 53.53 cm shoot length was recorded in *Trichoderma* isolate-IV @ 30 gm/kg soil which was statistically significant than the other treatments. Shoot length in *Trichoderma* isolate-V was recorded with 50.97 cm which was at par of *Trichoderma* isolate-III (49.84 cm) and *Trichoderma* isolate-II (49.69 cm) when applied as 30 gm/kg soil. In case of *Pseudomonas fluorescens* @ 10 ml/kg soil, average 33.62 cm shoot length was observed. Whereas, average 34.47 cm shoot length was observed in carbofuran @ 3gm/kg soil as compare to control (33.14 cm). It was observed that, fresh and dry shoot weight of rice plants was increasing as similar to length of rice plant. Maximum fresh and dry shoot weight (10.58 gm and 4.18 gm) was recorded in *Trichoderma* isolate-IV @ 30 gm/kg soil respectively. Fresh and dry shoot weight in all isolates of *Trichoderma* @ 30 gm/kg soil was differed significantly than compare to control. In case of *Pseudomonas fluorescens* @ 10 gm/kg soil, average 5.48 gm and 1.97 gm fresh and dry shoot weight was recorded respectively.

Data of root length indicates that , maximum 14.84 cm root length was recorded in *Trichoderma* isolate-V which was statistically at par with *Trichoderma* isolate-III (14.42 cm) and *Trichoderma* isolate-II (14.12 cm) when applied as 30 gm/kg soil but statistically significant than other treatments. In case of *Trichoderma* isolate-IV @ 30 gm/kg soil average 13.75 cm root length was observed followed by

Trichoderma isolate-V (12.81 cm), *Trichoderma* isolate-IV (12.50 cm) and *Trichoderma* isolate-II (12.14 cm) when applied as 20 gm/kg soil. In case of *Pseudomonas fluorescens* @ 10 ml/kg soil, average 9.15 cm root length was observed. Whereas, average 10.44 cm root length was observed in carbofuran @ 3gm/kg as compare of control (8.78 cm). At 40 days after seed sowing, maximum fresh and dry root weight (7.40 gm and 2.18 gm) was recorded in *Trichoderma* isolate-V @ 30 gm/kg soil respectively. Average 4.66 gm and 1.18 gm fresh and dry roots weight *Pseudomonas fluorescens* @ 10 ml/kg soil was recorded with respectively. Whereas, average 5.37 gm and 1.49 gm fresh and dry root weight was observed in carbofuran @ 3gm/kg as compare to control (4.05 gm and 1.22 gm) respectively.

Effect of *Trichoderma* isolates on J_2 of *M. graminicola*

Data of Table-2 indicates that at 24 hours after inoculation, maximum 80.83% larval mortality was recorded in *Pseudomonas fluorescens* suspension as compare to carbofuran in which 64.18% larval mortality was recorded. In *Trichoderma* isolates, maximum 45.83% larval mortality was recorded in *Trichoderma* isolate-IV followed by *Trichoderma* isolate-III (33.33%). In comparison of all treatments, control was recorded with 1.68% larval mortality. At 48 hours after inoculation, maximum 89.18% larval mortality was recorded in *Pseudomonas fluorescens* suspension. In case of carbofuran, 75.00% larval mortality was recorded. Among the *Trichoderma* isolates, maximum 70.00% larval mortality was recorded in *Trichoderma* isolate-IV followed by *Trichoderma* isolate-V (47.50%) and *Trichoderma* isolate-III (44.18%). At 72 hours after inoculation, maximum 96.68% larval mortality was recorded in *Pseudomonas fluorescens* suspension as compare to carbofuran (95.00%). Among *Trichoderma* isolates, maximum 74.18% larval mortality was recorded in *Trichoderma* isolate-IV followed by *Trichoderma* isolate-V and *Trichoderma* isolate-III in which 58.33% larval mortality was recorded as compare to control (4.18%).

The results of this study indicate that *Trichoderma* isolates significantly increased the shoot and root of rice plants. *Trichoderma* isolates were also reduced the infestation of root knot nematode. *Trichoderma* spp. have the significant effect on growth parameters and reduce the infestation of root knot nematode has been reported by many researchers in previous years. **Altmare *et al*. (1999)** have reported that in-cooperation of *Trichoderma* increase phosphate solubility and the availability of micro nutrients in the soil and it could promote growth of the plants. **Sharon *et al*. (2001)** reported that *T. harzianum* reduced galling of root-knot nematode, *M. javanica* on tomato plants. **Dababat and Sikora (2007)** used two species of *Trichoderma* (*T. viride* and *T. harzianum*) and found a significant reduction in tomato root galling infested with *M. incognita*. Similar result of reduction in infestation of nematode has reporded by **Pandey *et al.* (2003)** who used different treatments of *Trichoderma viride* against *M. incognita* in chickpea, in which all treatments of *T. viride* decreased galling.

Table 1. Effect of different bio-agents on plant growth parameters at 40 days after seed sowing

Treatment	Treatment	Doses (gm/kg)	Mean shoot length (cm)	Mean fresh shoot weight (gm)	Mean dry shoot weight (gm)	Mean root length (cm)	Mean fresh root weight (gm)	Mean dry root weight (gm)	Average galls/plant
T1	*Pseudomonas fluorescens*	10	33.62	5.48	1.97	9.15	4.66	1.18	8.00
T2	*Trichoderma* isolate-I	10	35.09	5.47	1.98	8.78	4.71	1.29	5.62
T3	*Trichoderma* isolate-I	20	42.50	6.45	2.31	10.30	5.13	1.36	5.89
T4	*Trichoderma* isolate-I	30	48.75	9.01	3.30	12.97	6.51	1.63	3.50
T5	*Trichoderma* isolate-II	10	36.14	6.03	2.28	10.53	5.08	1.37	6.89
T6	*Trichoderma* isolate-II	20	41.36	8.21	3.15	12.14	5.41	1.49	5.78
T7	*Trichoderma* isolate-II	30	49.69	9.64	4.02	14.12	6.99	1.81	3.06
T8	*Trichoderma* isolate-III	10	35.42	5.97	1.92	10.22	5.06	1.59	9.78
T9	*Trichoderma* isolate-III	20	44.28	8.77	3.34	10.95	5.82	1.76	7.28
T10	*Trichoderma* isolate-III	30	49.84	9.73	3.96	14.42	7.14	1.94	3.00
T11	*Trichoderma* isolate-IV	10	38.34	7.12	2.83	9.66	5.07	1.32	7.50
T12	*Trichoderma* isolate-IV	20	43.56	7.98	2.72	12.50	6.09	1.57	6.28
T13	*Trichoderma* isolate-IV	30	53.53	10.58	4.18	13.75	7.26	2.08	6.56
T14	*Trichoderma* isolate-V	10	39.06	7.57	2.77	11.53	5.48	1.18	6.34
T15	*Trichoderma* isolate-V	20	43.23	8.96	3.21	12.81	6.28	1.69	6.22
T16	*Trichoderma* isolate-V	30	50.97	10.10	4.13	14.84	7.40	2.18	1.61
T17	Carbofuran	3gm/kg	34.47	5.53	1.91	10.44	5.37	1.49	4.61

T18	Control		33.14	4.99	1.98	8.78	4.05	1.22	25.17
	C.D. at 5%		1.94	0.06	0.07	1.23	0.07	0.05	1.41

Table 2. Effect of filtrate of *Trichoderma* strains and *Pseudomonas fluorescens* on larval mortality of *M. graminicola*

Treatment	Treatment	Dead J_2 after 24 hours	Per cent mortality	Dead J_2 after 48 hours	Per cent mortality	Dead J_2 after 72 hours	Per cent mortality
T_1	*Trichoderma* isolat-I	3.67	9.18	11.33	28.33	18.33	45.83
T_2	*Trichoderma* isolate-II	4.33	10.83	13.67	34.18	21.00	52.50
T_3	*Trichoderma* isolate-III	13.33	33.33	17.67	44.18	23.33	58.33
T_4	*Trichoderma* isolate-IV	18.33	45.83	28.00	70.00	29.67	74.18
T_5	*Trichoderma* isolate-V	10.67	26.68	19.00	47.50	23.33	58.33
T_6	*Pseudomonas fluorescens*	32.33	80.83	35.67	89.18	38.67	96.68
T_7	Carbofuran	25.67	64.18	30.00	75.00	38.00	95.00
T_8	Control	0.67	1.68	1.33	3.33	1.67	4.18
	C.D. at 5% level	3.78		3.63		2.04	

ACKNOWLEDGMENT

Authors would like to extend our sincere thanks to Vice Chancellor of Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut for encouraging and providing necessary facilities to carry out this work.

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