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Efficacy of Fungal Bioagents for the Management of *Meloidogyne graminicola* Infecting Paddy

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ABSTRACT: The field experiments were carried out during summer, 2010 to 2015 to know the efficacy of bio agents viz., *Trichoderma viride, Paecilomyces lilacinus and Pochonia chlamydosporia* against root knot nematode, *Meloidogyne graminicola*. The bioagents were applied as seed treatment @10g/kg seed and in one treatment paddy seeds were treated with carbosulfan @ 3% w/w at the time of sowing. All the three bioagents were again applied in the field @ 2.5 kg/ha after 45 days of sowing of paddy seeds in the treatments where respective bioagents were applied earlier as seed treatment. In case of the treatment with Carbosulfan @ 3% w/w, Carbofuran was applied @ 1 kg *a.i.*/ha after 45 DAS. The data of all the years were pooled. The results revealed that all the treatments were significantly superior to untreated control with respect to nematode population, RKI, yield and ICBR. Treatment combination of seed treatment with Carbosulfan followed by soil application of Carbofuran at 45 DAS was most effective in reducing the nematode population, galls per plant and increasing yield (22.72 q/ha) except *T. viride* @10g/kg seed + soil application @2.5 kg/ha at 45 DAS which was at par in respect of yield. Among the bioagents, *T. viride* @10g/kg seed as seed treatment and soil application @ 2.5 kg/ ha at 45 DAS was found to be most effective in reducing nematode population, galls per plant and increasing yield (22.29 q/ha). The ICBR in *T. viride* @10g/kg seed as seed treatment + soil application @2.5 kg/ha at 45 DAS (1:4.96) was found to be more as compared to Carbosulfan as seed treatment followed by soil application of Carbofuran at 45 DAS (1:1.62). This was because of the lower cost of the bioagents as compared to the chemicals.

Key words: *Meloidogyne graminicola, Trichoderma viride, Paecilomyces lilacinus, Pochonia chlamydosporia,* Carbofuran, Carbosulfan

Asia is the main rice-growing region of the world, responsible for about 90% of global rice production, which is estimated at around 740 million tons of paddy rice annually (FAOSTAT, 2013). In addition, rice is the staple food for more than 50% of the population in Asia. In India, total rice production is 104.23 million tonnes in 2015-16 from an area of 43.86 million hectares. While in Assam the total area under rice is 2.28 million hectares with the production of 4.86 million tones in 2014-2015(Anonymous, 2015).

However, various pests and diseases which constitute important constraints in the successful crop production among which plant parasitic nematodes play an important role and more than 200 species of PPNs have been reported to be associated with rice (Prot, 1994). Out of the various plant parasitic nematodes, *Meloidogyne graminicola* is the dominant species infecting rice and is a serious pest of both cereals across rice–wheat rotation areas of South Asia's Indo-Gangetic plain and of rice producing areas of Southeast Asia. *M. graminicola* has been found to infect rice in different parts of India namely, Assam, Andhra Pradesh, Karnataka, West Bengal, Orissa, Kerala, Tripura and Madhya Pradesh (Prasad *et al*., 1987) and is a serious pest of upland rice and nurseries world over in well drained soils (Rao *et al*., 1986; Gaur & Pankaj, 2010; Pankaj *et al.*, 2010).

Field-based yield loss studies have indicated that the nematode can cause yield losses between 20 and 80% (Bridge and Page, 1982 and Padgham *et al*., 2004). Netscher and Erlan (1993) reported that *M. graminicola* caused 28 to 87% yield loss in upland rice in Indonesia. Jairajpuri and Baqri (1991) reported grain yield losses from 16 to 32%. Soriano *et al.* (2000) recorded 11 to 73% yield losses by this nematode under simulation of intermittently flooded rice, whereas under simulated upland conditions, yield loss varied between 20 and 98%

(Tandingan *et al*., 1996). Padgham *et al* (2004) also reported 16 to 20% yield loss caused by *M. graminicola* in low land rainfed rice in Bangladesh. In Assam, this nematode has been reported to cause an avoidable loss in yield to the tune of 20.60 per cent ((Anonymous, 2013).

Use of chemical nematicide is commonly adopted method for managing *M. graminicola* in rice. Several studies supported the effectiveness of carbofuran to control *M. graminicola* in rice (Dang-ngoc Kinh *et al*., 1982 and Prasad and Rao, 1985). However, nematode management strategies have moved towards partial or complete avoidance of chemicals due to groundwater contamination and other environmental hazards. This necessitates efforts to find alternative methods of nematode management in rice. Presently, field application of biological control agents is emerging as a promising alternative strategy in the management of nematodes and also regarded as an important component of integrated nematode management system. Among the various biocontrol agents reported to manage plant parasitic nematodes, the chitinolytic microbes such as *Trichoderma viride* (Priya, 2015) and egg parasitic fungi, *Purpureocillium lilacinum* (Thom.) Samson, appear to be ideal agents for the control of rice nematodes because they are known to survive better under clay soils (Seenivasan, 2011 and Priya, 2015) in which the irrigated rice is normally grown. Although soil nematicides are effective and fast-acting, they are currently being reappraised with respect to the environmental hazards and human health (Wachira *et al*., 2009). In addition to that, they are relatively unaffordable to many small scale farmers. Hence an eco-friendly and environmentally safe approach was aimed at through incorporation of bioagents in the management of root knot nematode, *Meloidogyne. graminicola* in direct seeded ahu rice under field conditions.

MATERIAL AND METHODS

The field experiments were carried out during summer, 2010 to 2015 as direct seeded ahu rice in a field naturally infested with *M. graminicola* at ICR Farm, Dept. of Nematology AAU Jorhat-13, Assam. The initial nematode population of *M. graminicola* during the years recorded to be 228 (2010), 216 (2011), 217 (2012), 212 (2013) 224 (2014) and 216 (2015) J2/200 cc of soil. The susceptible variety Luit was used for the studies. The experiments were laid out in a randomized block design (RBD) with five treatments replicated four times. The bioagents were used both as seed treatment as well as soil application. The treatments were: T_1- Untreated control, T_2- *T. viride* @10g/kg seed + soil application @2.5 kg/ha at 45 DAS, T_3- *P. chlamydosporia* @10g/kg seed + soil application @2.5 kg/ha at 45 DAS, T_4- *P. lilacinus* @10g/kg seed + soil application @2.5 kg/ha at 45 DAS and T_5- Seed treatment with Carbosulfan @ 3% w/w + soil application of Carbofuran @1 kg *a.i*./ha at 45 DAS. *T. viride, P. chlamydosporia* and *P. lilacinus* @ 10g/kg of seed were applied as seed treatment at the time of sowing and in one treatment paddy seeds were treated with carbosulfan @ 3% w/w at the time of sowing. All the three bioagents were again applied in the field @ 2.5 kg/ha after 45 days of sowing of paddy seeds in the treatments where respective bioagents were applied earlier as seed treatment. The bioagents were applied to the soil in the form of FYM after enriching with the bioagents @ 1% w/w. For this, the required amount of bioagent was mixed with well dried FYM, moistened and incubated for 10 days by covering with a gunny sheet. Thereafter, it was mixed thoroughly before applying in the soil. In case of seeds treated with carbosulfan @ 3% w/w, carbofuran was applied @ 1 kg *a.i*./ha after 45 DAS. The observation on final nematode population in 200 cc soil and in 5g root, number of galls/root system and grain yield per plot were recorded and incremental cost benefit ratios (ICBR) in relevant treatments were calculated. The soil population of juveniles of *M. graminicola* was determined using modified Cobb's sieving and decanting technique and root knot index at harvest was recorded according to the number of galls per root system Taylor and Sasser (1978). The data of all the years were pooled and subjected analysis of variance (ANOVA), using Microsoft office Excel Worksheet, and significant means separated, using critical difference (CD) at 5%.

RESULTS AND DISCUSSION

From the results of pooled data (Summer, 2010 to 2015) as presented in Table 1 and Table 2 it was observed that seed treatment with carbosulfan followed by soil application of Carbofuran at 45 DAS (T_5) was most effective in reducing the soil and root nematode population (49.94 % and 45.58 % respectively), root knot index (RKI) (28.75 %) and increasing yield (23.28 %) as compared to untreated control (T_1). This treatment was significantly superior to rest of the treatments except the treatment, *T. viride* @10g/kg seed + soil application @2.5 kg/ha at 45 DAS (T_2) which was at par. There was decrease in soil and root nematode population (45.96 % and 40.25 % respectively), RKI (25.75 %) and increase in yield (20.94 %) as compared to untreated control (T_1). The yield in the treatments, T_5 and T_2 were recorded to be 22.72 q/ha and 22.29 q/ha respectively as against 18.43 q/ha in untreated control (T_1). Although the yields in the treatments T_2 and T_5 were at par, the ICBR in T_2 (1:4.96) was found to be much higher as compared to the treatment T_5 (1:1.62). This was because of the lower cost of the bioagents as compared to the chemicals.

Although the chemical nematicides are always found to provide quick and better control of plant parasitic nematodes, because of their adverse effect on human health and environment there has been growing reluctance for its use. On the other hand, the bioagents are self propagating under favorable conditions and therefore may remain in soil for long period and also produce enzymes such as chitinases which are capable of rupturing nematode egg shells that result in reduced multiplication of nematodes (Gortari and Hours, 2008). *T. viride* is known and considered as an economically viable and ecofriendly alternative to chemical nematicides against root knot nematode in various crops (Pathak *et al*., 2005). The present study indicated all the bioagents to be effective against *M. graminicola, T. viride* was found to be the best and was comparable to the chemicals. Priya (2015) reported that soil application of *T. viride*

Table1. Efficacy of fungal bioagents against *M. graminicola* on rice, (Pool of summer, 2010 to 2015)

Treatment	Galls in 20 seedlings	% decrease over control	Nematode population			
			Soil (200cc)	% decrease over control	Root (5g)	% decrease over control
T1	85.33		285.00		81.17	
T2	51.00	40.23	154.33	45.96	48.50	40.25
T3	56.17	34.17	174.67	38.71	57.17	29.57
T4	55.00	35.54	165.83	41.81	52.50	35.21
T5	44.67	47.65	142.67	49.94	44.17	45.58
S. Ed ±	2.35		5.93		2.62	
CD (0.05)	5.09		12.86		5.68	

Table2. Efficacy of fungal bioagents against *M.graminicola* on rice, (Pool of summer, 2010 to 2015)

Treatment	RKI at harvest	% decrease over control	q/ha	% increase over control	ICBR
T1	4.00		18.43		-
T2	2.97	25.75	22.29	20.94	1:4.96
T3	3.37	15.75	20.69	12.26	1:2.82
T4	3.15	21.25	20.86	13.18	1:3.11
T5	2.85	28.75	22.72	23.28	1:1.62
S. Ed ±	0.18		0.52		
CD (0.05)	0.39		1.13		

and *P. fluorescens* increased the yield of *Withania somnifera* and were found to be at par with carbofuran. In addition to direct antagonism, the biocontrol agents increase the activity of various defense-related enzymes and chemicals in response to pathogen infection. All plants are known to be endowed with defense genes, which are quiescent in nature and require the appropriate stimulation signals to activate them. It has been reported that biocontrol agents trigger/activate latent plant defense mechanisms in response to pathogen infection. Inducing the plant's own defense mechanism by applying biological agents is a novel strategy in nematode management. Application of bioagents was found to increase chitinase enzyme accumulation in rice plants (Vidyasekaran *et al.* 2001, Nandakumar *et al.* 2007 and Seenivasan, 2011). Chitinase is a hydrolytic enzyme that degrades chitin (a polymer of β-1,4-linked N-acetylglucosamine), a structural component of nematode egg shells (Muzzarelli, 1977). Plant chitinase have been proposed to play an important role in defense against plant parasitic nematodes (Cohn and Spiegel, 1991). Qiu *et al.* (1997) also reported that higher chitinase activity in roots was associated with resistance to *M. incognita* in soybean. Annapurna *et al*. (2018) reported increase in defense related enzymes such as peroxidase, polyphenol oxidase and phenyl alanine amoniaa lyase and total phenol in tomato plants inoculated with *T. harzianum*, *Purpureocillium lilacinum* and *Pochonia chlamydosporia* in tomato plants against *M. incognita*. They also reported highest enzymatic activity in plants inoculated with *T. harzianum*. Similar mode of action of the bioagents might be operative in the present investigation resulting in decreased nematode infection and multiplication with increased yield. In addition, the plant growth regulators, including gibberellins, cytokinins and indole – 3 acetic acid (IAA) produced by bioagents also reported to constitute a mechanism for plant growth promotion (Brown, 1974 and Lifshits *et al*., 1987). In the present investigation, it was observed that among the bioagents, *Trichoderma viride* @10g/kg seed as seed treatment and soil application @ 2.5 kg/ha at 45 DAS was found to be most effective in reducing nematode population, galls per plant and increasing yield (22.29 q/ha) with a favorable ICBR in ahu rice under Assam condition.

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