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INTEGRATED MANAGEMENT OF RICE ROOT-KNOT NEMATODE, *MELOIDOGYNE GRAMINICOLA* UNDER THE DIRECT-SEEDED CONDITION

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ABSTRACT

The present field experiment was conducted to determine the efficacy of consortium of different bio-agents viz., *Psuedomonas fluorescens + Trichoderma harzianum + Bacillus megatherium,* organic amendments viz., neem cake, poultry manure and nematicides viz., carbosulfan, carbofuran and fluensulfone for the management of *M. graminicola* under direct-seeded condition during kharif 2019-20 at the University of Agricultural and Horticultural Sciences, Shivamogga. The results revealed that all the treatments were significantly superior over the untreated check with respect to plant growth parameters and nematode population. However, the plots treated with fluensulfone at 3g/plot was found to be the best treatment as it recorded highest plant height (78.87 cm), root length (18.90 cm) with lowest RKI (2.0), maximum grain yield (36.87 q/ha) and least nematode population (199.00/200g soil) followed by the consortium of bioagents *P. fluorescens + T. harzianum + B. megatherium* at 20g/m2, carbofuran 3G at 9.9g/m2, carbosulfan 25 EC at 0.1%, neem cake at 100g/m2 and poultry manure at 100g/m2 respectively.

Keywords: management, *Meloidogyne graminicola*, rice root-knot nematode, direct-seeded rice

INTRODUCTION

Rice (*Oryza sativa)* is the seed of grass species as a cereal grain, the most widely consumed staple food for a two-third of world human population grown in more than a hundred countries. The unique feature of rice cultivation in the state is that it can be sown or transplanted at all seasons of the year. The projection of India's rice production target for 2025 AD is 140mt, which can only be achieved by increasing rice production by more than 2mt per year over the next decade, and this must be achieved against a back-drop of declining natural resources such as land and water. The expected future demand for rice from the increased population can no longer be met only by higher yields from irrigated areas, but also rainfed conditions (Ravindra *et al*., 2014). By 2025, water scarcity will threaten 30 percent of the human population (Anon, 2003). Among other challenges, the ongoing lockdown due to the COVID-19 pandemic is likely to create a labour shortage problem in agriculture due to the unavailability of migrant farm workers. The existing practice of transplanted rice (TPR) cultivation over the entire available area will be very difficult to follow. Due to labour shortages in the three granary states of Punjab, Haryana and Jammu farmers are now encouraged to adopt 'Direct Seeding of Rice' (DSR) instead of conventional transplantation (Pawan *et al*., 2020). The direct-seeded rice production system is a relatively new production system in which specially developed rice cultivars are established in non-puddled, non-flooded, non-saturated fields and cultivated under conditions similar to upland conditions through direct seeding (Zhao *et al*., 2010). The crop, rice is suffered due to the number of microflora that includes bacteria, fungi, nematode, virus and mycoplasma. It is susceptible to root-knot nematodes and is attacked by *M. incognita*, *M. graminicola*, *M. triticoryzae, M. javanica, M. oryzae* and *M. arenaria* (Gaur *et al*., 2010). Among such species, *M. graminicola* is a primary pest of rice. It poses a significant threat to rice cultivation, especially in Southeast Asia, where around 90 % of the world's rice is produced and consumed (Dutta *et al*., 2012). *M. graminicola* shows yellowing, dwarfing and gall formation on the roots of rice plants. The degree of manifestation of symptoms varies with the time of infection, the age of the plants and the inoculum load (Upadhyay *et al*., 2014). Rice root-knot nematode, *M. graminicola* Golden and Birchfield 1965 has emerged as a pest of international importance (Waele *et al*., 2007) . Under the simulation of intermittently flooded rice, *M. graminicola* reported causing 11 to 73 per cent yield losses whereas, under simulated upland conditions, yield loss varied between 20 and 98 per cent (Soriano *et al*., 2000) . Because of the enormity of the yield losses in direct-seeded rice caused by rice root-knot nematodes, it is necessary to minimize crop damage by adopting integrated nematode management strategies. Since *M. graminicola* causes severe yield losses, efforts in the recent past have been initiated on the management of this nematode through host resistance, bio-agents and chemicals. A sufficient amount of work has been done on various aspects of pathogen and its management against rice root-knot nematode in submerged rice. So far more studies have not been carried out or reported on aspects of the availability of tolerant or resistant cultivars and effective, eco-friendly management of root-knot nematode in direct-seeded rice. Keeping these points in view, the present investigation was undertaken with integrated management of rice root-knot nematode, *M. graminicola* under direct-seeded condition.

MATERIALS AND METHODS

The experiment was laid out in rice root-knot nematode, *M. graminicola* sick soil (sandy loam soil) at the College of Agriculture, UAHS, Shivamogga during *kharif*-2019 to know the effect of integrated management of nematode practice on rice root-knot nematode. For the study, the susceptible variety of Jyothi was used. Before conducting the experiment, the land was thoroughly ploughed with a tractor driven disc plough followed by harrowing. Then the stubbles and weeds were removed from the field. The land was then ready for sowing of rice seeds. The layout of the experimental field was done according to the design.

Treatment details

T_1 = Seed treatment with carbosulfan 25EC at 0.1% seed

T_2 = Soil drenching with carbofuran 3G at 9.9 g/m^2 (1 kg a.i./ha)

T_3 = Application of consortia of bioagents, *Pseudomonas fluorescens* (CFU 1 x 10^8/g) at 20 g/m² + *Trichoderma harzianum* (CFU 2 × 10^6/g) at 20 g/m² + *Bacillus megatherium* (CFU 1x10^8/g) at 20 g/m^2

T_4 = Application of neem cake at 100 g/m^2

T_5 = Application of poultry manure at 100 g/m^2

T_6 = Application of nematicide fluensulfone at 3 g/plot

T_7 = Untreated control

The observations were recorded on plant growth parameters such as plant height (cm), root weight (g), root length (cm), and grain yield per plot, Root-Knot Index, nematode populations in 200cc soil, number of galls/ root system. The soil population of *M. graminicola* was determined using Cobb's decanting and sieving method (modified), followed by Baermann's funnel technique (Southey *et al*., 1986) and the root knot index was recorded based on 0-5 rating scale according to the number of galls per root system in which 0=No galls (Immune), 1=1-2 galls/root system (Resistant), 2=3-10 galls/root system(Moderately resistant) 3=11-30 galls/root system(Moderately susceptible) 4=31-100 galls/root system (Susceptible) and 5=>100 galls/ root system(Highly susceptible) Taylor and Sasser (1978).

Statistical analysis: Statistical analyses for *in-vivo* studies were performed on the data obtained in the present investigation concerning parameters such as plant height (cm), root length (cm), root weight (g) and grain yield per plot, nematode populations in 200cc soil, gall/root system number and egg mass/root system number.

RESULTS AND DISCUSSION

The present study revealed that all treatments concerning plant growth parameters and nematode population were significantly higher than the untreated control. The results obtained from the current study are given in Tables 1, 2, 3 and 4.

Effect of treatments on plant growth parameters of rice

Effect on Plant height: The plant height of rice differed significantly in various treatments, and all the treatments were significantly higher than untreated control (16.53 cm). The plots treated with fluensulfone was recorded with higher plant height (26.13 cm) followed by the consortium of bioagents *P. fluorescens* + *T. harzianum* + *B. megatherium* (23.40 cm), carbofuran 3G (22.13 cm) and carbosulfan (21.13 cm). The plots that were treated with neem cake and poultry manure recorded the plant height of (19.73 cm) and (19.00 cm) respectively. The similar trends were observed at 60, 90 days after sowing and at the time harvest (Table.1, Fig.1).

Effect on Root length: The length of the root differed significantly in various treatments. All treatments had a root length greater than the untreated control. The effect of applying fluensulfone to the soil showed the highest root length (18.90 cm) followed by a consortium of bioagents *P. fluorescens* + *T. harzianum* + *B. megatherium* (17.30 cm) which was followed by carbofuran 3G (16.63 cm), carbosulfan (15.97 cm) neem cake (13.43 cm) and poultry manure (12.27 cm) respectively. The minimum root length (7.17 cm) was observed in the control plot. With respect to root weight, fluensulfone recorded highest fresh weight (7.46 gm) and dry weight (3.92 gm) followed by the consortium of bioagents, *P. fluorescens* + *T. harzianum* + *B. megatherium* fresh weight (7.10gm) dry weight (3.52 gm), carbofuran 3G fresh weight (6.79 gm) dry weight(3.22 gm), carbosulfan fresh weight (6.39 gm) dry weight (2.92 gm), neem cake fresh weight (5.93 gm) dry weight (2.61 gm), poultry manure fresh weight (5.75 gm) dry weight (2.28 gm), respectively. However, the least root weight was observed in untreated control fresh weight (5.03 gm) dry weight (1.94 gm). (Table. 1)

Effect on grain yield and RKI: Data on the efficacy of a consortium of bio-agents, organic amendments and nematicides on the rice grain yield was recorded at the time of harvesting are presented in table 2, Fig.2.

All the treatments recorded significantly higher

Plate 2. Effect of bio-agents, nematicides and organic amendments on plant growth parameters of rice infested with rice root-knot nematode, M. graminicola

Table 1: Effect of the consortium of bio-agents, organic amendments and nematicides on plant growth parameters of rice infested by *M. graminicola*

Treatment	**Plant height(cm)**				**Root length (cm)**	**Root weight (gm)**	
	30DAS	**60DAS**	**90DAS**	**time of harvest**		**Fresh**	**Dry**
T1= Seed treatment with carbosulfan 25 EC at 0.1%	21.13 (4.63)*	15.97 (4.03)*	55.53 (7.46)	68.67 (8.31)	15.97 (4.03)*	6.39 (2.62)	2.92 (1.85)
T2= Carbofuran 3G at 9.9g/m2	22.13 (4.74)	16.63 (4.13)	58.07 (7.63)	75.27 (8.70)	16.63 (4.13)	6.79 (2.69)	3.22 (1.93)
T3= Consortia of bioagents- Pseudomonas flourescens + Trichoderma harzianum+ Bacillus megatherium at 20g/m2	23.40 (4.87)	17.30 (4.21)	60.20 (7.76)	77.03 (8.79)	17.30 (4.21)	7.10 (2.75)	3.520 (2.00)
T4= Neem cake at 100g/m2	19.73 (4.48)	13.43 (3.71)	46.37 (6.81)	61.27 (7.85)	13.43 (3.71)	5.93 (2.53)	2.61 (1.76)
T5= Poultry manure at 100g/m2	19.00 (4.38)	12.27 (3.53)	45.53 (6.75)	60.33 (7.80)	12.27 (3.53)	5.75 (2.50)	2.28 (1.66)
T6= Fluensulfone at 3g/plot	26.13 (5.16)	18.90 (4.40)	62.60 (7.91)	78.87 (8.90)	18.90 (4.40)	7.46 (2.82)	3.92 (2.10)
T7=Untreated control	16.53 (4.11)	7.17 (2.75)	41.67 (6.45)	57.20 (7.59)	7.17 (2.75)	5.03 (2.34)	1.94 (1.56)
S. Em.±	0.134	0.256	0.048	0.284	0.256	0.096	0.081
CD (P=0.05)	0.414	0.790	0.149	0.876	0.790	0.299	0.2483

* Figures in the parenthesis are square root transformed value
DAS= Days After Sowing

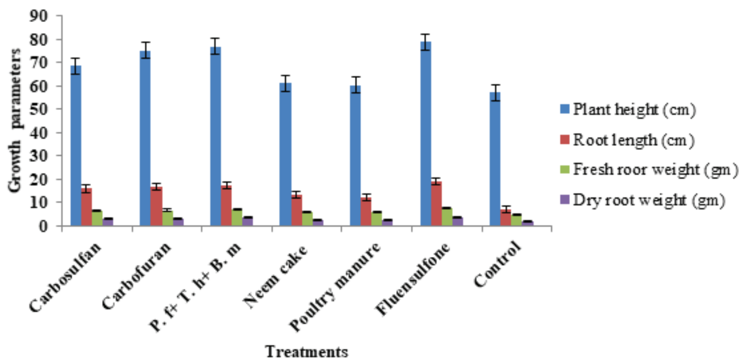

Fig 1: Effect of the consortium of bio-agents, organic amendments and nematicides on plant growth parameters of rice infested by *M. graminicola*

yield and lowest RKI compared with the untreated checks. Rice yield per plot was considerably higher in all treatments compared to untreated control. Maximum yield was recorded in fluensulfone treated plots (36.87 q/ha) with least Root-Knot Index (2.0) followed by bioagent's consortium *viz*., *P. fluorescens* + *T. harzianum* + *B. megatherium* (35.20 q/ha) with RKI (2.3), carbofuran (33.33 q/ha) with RKI (2.6) and carbosulfan (30.73 q/ha) with RKI (3.0) which was followed by neem cake (27.23 q/ha) with RKI (3.3) and poultry manure (26.70 q/ha) with RKI (3.3). While in untreated control, the least yield (21.93 q/ha) and maximum RKI (4.0) were observed.

Effect on Nematode population in soil: The observation was recorded after the harvest of the crop with respect to nematode population in soil revealed that fluensufone was significantly superior compared to rest of the treatments and recorded least nematode population (199.00) followed by the consortium of bioagents *viz*., *P. fluorescens* + *T. harzianum* + *B. megatherium* (213.00), carbofuran 3G (242.00), carbosulfan (278.00) neem cake (309.00) and poultry manure (320.00) however, the highest nematode population was observed in the control plot (586.00) (Table 3, Fig.3).

All the treatments were significantly superior compared to the control plot. Among the tested

Table 2: Effect of the consortium of bio-agents, organic amendments and nematicides on the yield of rice and root-knot index of rice plants infested with rice root-knot nematode, *M. graminicola*

Treatments	Yield (q/ha)	RKI
T1= Seed treatment with carbosulfan 25 EC at 0.1%	30.73 (5.59)*	3.0
T2= Carbofuran 3G at 9.9g/m2	33.33 (5.81)	2.6
T3=Consortia of bioagents-Pseudomonas flourescens+ Trichoderma harzianum + Bacillus megatherium at 20g/m2	35.20 (5.97)	2.3
T4= Neem cake at 100g/m2	27.23 (5.25)	3.3
T5= Poultry manure at 100g/m2	26.70 (5.19)	3.3
T6= Fluensulfone at 3g/plot	36.87 (6.11)	2.0
T7=Untreated control	21.93 (4.73)	4.0
S. Em.±	0.231	-
CD (P=0.05)	0.713	-

* Figures in the parenthesis are square root transformed value

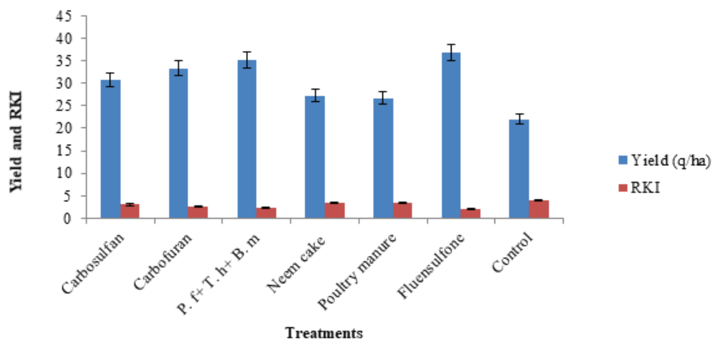

Fig 2. Effect of the consortium of bio-agents, organic amendments and nematicides on yield and root-knot index of rice infested by *M. graminicola*

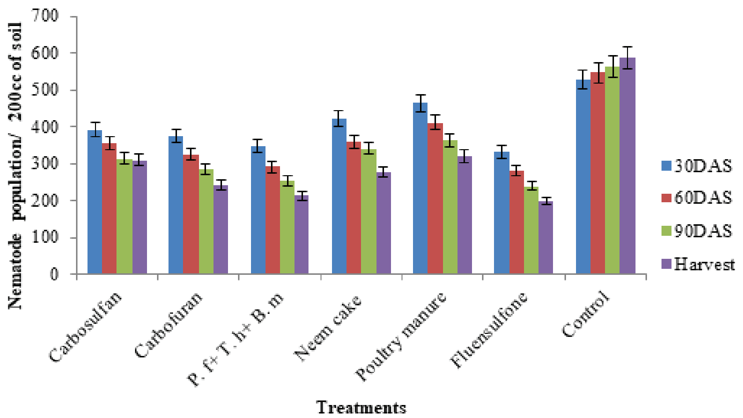

Fig 3. Effect of consortium of bio-agents, organic amendments and nematicides on nematode population of M. graminicola

treatments the least nematode population was recorded in fluensulfone, a consortium of *P. fluorescens* + *T. harzianum* + *B. megatherium*, carbofuran and carbosulfan these were found to be effective for the management of rice root-knot nematode.

Effect on number of galls and egg masses: The data obtained on the number of galls and egg masses showed that all treatments were significantly superior over untreated control. The plot treated with fluensulfone showed least galls and egg masses 5.33 and 5.40 followed by the consortium of *P. fluorescens* + *T. harzianum* + *B. megatherium* 9.33 galls 6.93 egg masses, carbofuran 13.00 galls 8.67 egg masses, carbosulfan 16.67 galls 10.50 egg mases, neem cake 21.33 galls 11.43 egg masses and poultry manure 24.33 galls 12.60 egg masses respectively (Table 4, fig. 4). It was evident from the above findings that all the treatments were effective in reducing the nematode population and increasing yield and growth parameter of rice in comparison to control. Fluenulfone was found to be the most effective treatment for the control of rice root-knot nematode followed by the consortium of *P. fluorescens* + *T. harzianum* + *B. megatherium,* carbofuran, carbosulfan, neem cake and poultry manure.

The present results are in conformity with the findings of (Oka *et al.,* 2012) who found the efficacy of fluensulfone in reducing the galling index and number of nematode eggs in the roots. Similar results were also reported by (Paul *et al.*, 2019), (Oka *et al.,* 2019) who reported that the use of fluensulfone could effectively manage the population of root-knot nematode, *M. graminicola*. (Narasimhamurthy *et al.,* 2017) who reported that consortium of *P. fluorescens* + *T. harzianum* was found to be the most effective treatment as it had the highest plant height, root length, maximum yield of grain with the lowest RKI and the lowest population of nematodes. (Priya *et al.*, 2015) reported that *Trichoderma viride* had given lowest nematode population, least galling, and higher yield followed by *Pseudomonas fluorescens* and *Bacillus subtillis*. Application of Carbofuran to the soil in the nursery and main field at the rate of 1kg a.i. / ha decreased *M. graminicola* population by over 90 per cent and resulted in increased yield of about 100 per cent (Anon, 1985). (Padgham *et al.*, 2005) and (Anita *et al.*, 2012) who reported that induction of phenol, polyphenol oxidase, peroxidase, superoxide dismutase, phenyl ammonia-lyase and chitinase to *M. graminicola* resulting in

Table 3: Effect of consortium of bio-agents, organic amendments and nematicides on nematode population of soil infested with rice root-knot nematode, *Meloidogyne graminicola*

Treatment	Nematode population in 200 cc of soil			
	30DAS	60DAS	90DAS	time of harvest
T1= Seed treatment with carbosulfan 25 EC at 0.1%	391.00 (19.77)*	353.00 (18.77)	313.00 (17.70)	278.00 (16.69)
T2= Carbofuran 3G at 9.9g/m2	374.00 (19.34)	325.00 (18.03)	285.00 (16.85)	242.00 (15.49)
T3= Consortia of bioagents- Pseudomonas flourescens + Trichoderma harzianum+ Bacillus megatherium at 20g/m2	346.00 (18.64)	291.00 (17.04)	253.00 (15.89)	213.00 (14.55)
T4= Neem cake at 100g/m2	421.00 (20.51)	360.00 (18.97)	341.00 (18.47)	309.00 (17.57)
T5= Poultry manure at 100g/m2	463.00 (21.52)	412.00 (20.29)	364.00 (19.08)	320.00 (17.87)
T6= Fluensulfone at 3g/plot	331.00 (18.19)	281.00 (16.75)	238.00 (15.42)	199.00 (14.08)
T7=Untreated control	529.00 (22.99)	547.00 (23.39)	563.00 (23.74)	586.00 (24.22)
S. Em.±	0.583	0.650	0.602	0.595
CD (P=0.05)	1.797	2.002	1.856	1.835

* Figures in the parenthesis are square root transformed value
DAS= Days after sowing; Average INP=480 J2 / 200cc soil

Table 4. Effect of consortium of bio-agents, organic amendments and nematicides on number of galls and number of egg masses/ root system under *in vivo* condition

Treatments	Number of galls/root system	No. of egg masses / root system
T1 = Seed treatment with carbosulfan 25 EC at 0.1%	16.67 (4.14)*	10.50 (3.30)
T2 = Carbofuran 3G at 9.9g/m2	13.00 (3.65)	8.67 (2.99)
T3 = Consortia of bioagents- Pseudomonas flourescens +Trichoderma harzianum+ Bacillus megatherium at 20g/m2	9.33 (3.13)	6.93 (2.72)
T4 = Neem cake at 100g/m2	21.33 (4.67)	11.43 (3.44)
T5 = Poultry manure at 100g/m2	24.33 (4.98)	12.60 (3.60)
T6 = Fluensulfone at 3g/plot	5.33 (2.41)	5.40 (2.40)
T7 =Untreated control	30.00 (5.52)	13.27 (3.71)
S. Em±	0.151	0.205
CD (P=0.05)	0.464	0.633

* Figures in the parenthesis are square root transformed value

significant reduction of the nematode infection. Integrated nematode management technology has resulted in the reduction of the nematode population and increased yields (Somasekhara *et al*., 2012). (Sehgal *et al*. 2014) gave that application of carbofuran, *P. fluorescens* and *T. viride* to nursery bed reduces the galls increases yield. The reason for the increase in growth parameters is due to the effect of flensulfone on the inhibition of J_2 mobility within the roots, as well as the formation of feeding sites. Sometimes fluensulfone might arrest J_1 by inhibiting feeding; thus; J_1 could enter the diapause and could not able to get moulted to the next larval stage, which leads to a reduction in the population of nematodes.

Considering the performance of the mixture of compatible biocontrol agents, *T. harzianum, P. fluorescens* and *B. megatherium* would more closely mimic the natural situation. It could broaden the spectrum of biocontrol activities with increased efficacy. They also act as growth-promoting organisms as they increase plant height, root length and yield by reducing the nematode population and serve as nematophagous fungi by producing a unique structure that kills eggs and juveniles by producing toxins and alkaloids that hinder the growth and activity of

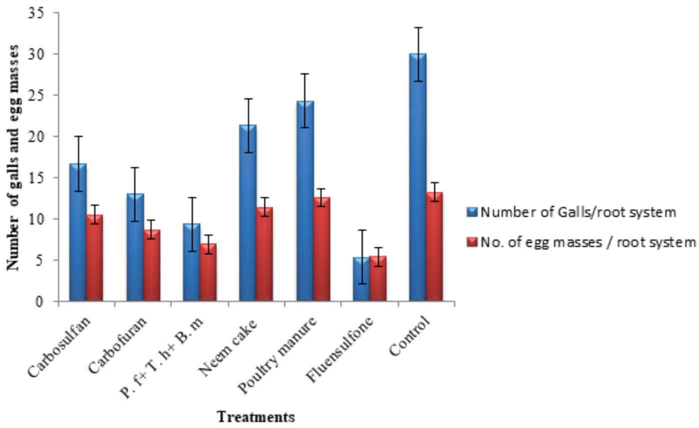

Fig 4. Effect of consortium of bio-agents, organic amendments and nematicides on number of galls and egg masses of M. graminicola

nematodes (Siddiqui *et al*., 2004). The other methods of nematode management such as field sanitation, fallowing, flooding, crop rotation and resistant crop varieties are having their limitations and majority of the times not practicable.

CONCLUSION

The present study of integrated nematode management in the direct-seeded rice revealed that among the different treatments, fluensulfone showed a maximum reduction of nematode population in soil and also resulted in the prevention of galling and egg mass production with a corresponding increase in plant growth parameters and also yield. This could be due to the inhibition of J_2 mobility within the roots, as well as the formation of feeding sites. Sometimes fluensulfone might arrest J_1 by inhibiting feeding; thus; J_1 could enter the diapause and could not able to get moulted to the next larval stage, which leads to a reduction in the population of nematodes.

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