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Narasimhamurthy HB
Department of Plant Pathology, College of Agriculture, Karnataka, India

Ravindra H
All India Coordinated Research Project (Nematodes), Karnataka, India

Mukesh Sehgal
ICAR-National Research Centre for Integrated Pest Management, LBS, Building, Pusa Campus, New Delhi, India

Suresha D Ekabote
Department Crop Protection, College of Agriculture, Hiriyuru-, UAHS, Shivamogga, Karnataka, India

Ganapathi
Organic Farming Research Centre, University of Agricultural and Horticultural Science, Shivamogga, Karnataka, India

Correspondence
Narasimhamurthy HB
Department of Plant Pathology, College of Agriculture, UAHS Karnataka, India

Bio-management of rice root-knot nematode (*Meloidogyne graminicola)*

Narasimhamurthy HB, Ravindra H, Mukesh Sehgal, Suresha D Ekabote and Ganapathi

Abstract
The present field experiments was conducted to know the efficacy of bio agents *viz., Paecilomyces lilacinus, Pseudomonas fluorescens, Pochonia chlamydosporia, Trichoderma harzianum, Bacillus subtilis,* Consortium of bio-agents (*Pseudomonas fluorescens* + *Trichoderma harzianum*) and Carbofuran 3G @ 0.3 ai. alone for management of rice root-knot nematode *Meloidogyne graminicola* during *Kharif*-2015 at College of Agriculture, Shivamogga (Latitude: 13° 27'-14° 39' N, Langitude: 74° 37'-75° 52' E). The results revealed that all the treatments were significantly superior over check with respect to growth parameters and nematode population. However, the treatment combination of *P. fluorescens* @20g/m² + *T. harzianum* @ 20g/m² was found to be the best treatment as it recorded highest plant height (83.33 cm), root length (21.27 cm), maximum grain yield (45.60 q/ha) with least RKI (1.20) and least nematode population (185.44/200g soil) with reduction of 72.77% nematode population followed by Carbofuran 3G @ 0.3 ai /m², *T. harzianum* @ 20 g/m², *P. fluorescens* @ 20 g/m² and *B. subtilis* @ 20 g/m² respectively.

Keywords: biomanagement, *Meloidogyne graminicola*, Rice, Rice root-knot nematode

1. Introduction
Rice (*Oryza sativa* L.) is one of the most important staple food crops of India and is a major source of calories for about 60 per cent of world population and influences the livelihoods and economies of several billion people especially concentrated in Asia, Latin America, Middle East, and the West Indies. For centuries, rice has shaped Asian societies and their cultures. Asian cultures are partly cultures of rice and many Asian societies relate to rice beyond the satisfaction of basic needs. It is cultivated in five major ecosystems *viz.*, irrigated, deep water, upland, low land and rainfed rice. About 53% of the world's rice is grown under irrigated conditions that provide 75% of total global production. Rainfed lowland rice (31% of the world rice area) is entirely dependent on rainfall, whereas, the deep water area (35%) occurs in the river deltas. Upland rice area (13%) is also rainfed but without surface water accumulation [1]. It is affected by several biotic and abiotic stresses, of which, plant parasitic nematodes constitute an important component [2]. Over 200 species of plant parasitic nematodes have been reported to be associated with rice [3] and are becoming increasingly important in the rapidly changing production system of rice [4]. It is susceptible to root-knot nematodes and is attacked by *M. incognita, M. graminicola, M. triticoryzae, M. javanica, M. oryzae* and *M. arenaria* [5]. 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 [6]. *M. graminicola* causes terminal, hook shaped or spiral galls which are characteristic symptoms of the infection of this nematode species [7]. Rice root-knot nematode, *M. graminicola* Golden and Birchfield 1965 has emerged as a pest of international importance [8]. *M. graminicola* reported to cause 11 to 73 per cent yield losses by this nematode under simulation of intermittently flooded rice, whereas under simulated upland conditions, yield loss varied between 20 and 98 per cent [9]. In view of the enormity of the yield losses caused by rice root-knot nematodes in rice, it is necessary to minimize crop damage by adopting available environment friendly management methods. Therefore, environmentally friendly alternatives are required for nematode control. There are various methods of nematode management that may prove effective against rice root-knot nematodes. Despite the known deleterious effects of chemicals, pesticides are still the most effective means of nematode management in rice ecosystems [7].

Several efforts for managing root knot nematode using chemicals are not satisfactory to control; cost of chemicals and residue problems has made the nematode management strategy unattractive for the growers and extension specialists. Chemicalisation of agro ecosystem depleted soil biota and withdrawal soil antagonists and beneficial organisms in soil environment promoted harmful plant pathogens including phytoparasitic nematodes. 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 [10]. *Pseudomonas fluorescens* and *Trichoderma* spp. are among the most commonly used biocontrol agents (BCAs) against plant parasitic nematodes [11, 12]. In addition to the suppressive action against target pathogens, the application of these biocontrol agents triggers or activates latent defense mechanisms in plants [13]. The present study was conducted to know the efficacy of bio agents for the management of rice root-knot nematode under field condition.

2. Materials and Methods

The present experiment was conducted in the month of June, *Kharif*-2015 in a field naturally infested with *M. graminicola* at College of Agriculture, Zonal Agricultural and Horticultural Research Station, Shivamogga (13° 27'- 14° 39' North latitude and 74° 37'- 75° 52' East Longitude with an altitude of 650 meters above the mean sea level. Karnataka, India). The experiment was laid out in a randomized complete block design (RCBD) by maintaining eight treatments with three replication. The susceptible variety Jyothi was used for this study and twenty four day old seedlings were transplanted in the field using two seedlings/ hill with a spacing of 20 x20 cm. The crop was transplanted during 3rd week of June.

2.1. Treatment details:

It includes different bioagents *viz., Paecilomyces lilacinus* @20g/m², *Pseudomonas fluorescens* @20g/m², *Pochonia chlamydosporia* @20g/m², *Trichoderma harzianum* @20g/m², *Bacillus subtilis* @20g/m², consortium of *P. fluorescens* @20g/m² + *T. harzianum* @20g/m², Carbofuran 3G @0.3 a.i /m² and Untreated control.

The observation on plant growth parameters such as plant height (cm), root length (cm), root weight (g) and grain yield per plot, Root Knot Index, nematode populations in 200cc soil, number of galls/root system were recorded. The soil population of *M. graminicola* was determined using Cobb's decanting and sieving method (modified), followed by Baermann's funnel technique [14] and 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) [15].

2.2. Statistical analysis

The data obtained in the present investigation regarding parameters such as plant height (cm), root length (cm), root weight (g) and grain yield per plot, nematode populations in 200cc soil, number of galls/root system and number of egg masses/ root system were subjected to statistical analyses for *in-vivo* studies.

3. Results and Discussion

The present study results revealed that all the treatments were significantly superior over untreated check with respect to plant growth parameters and nematode population. The results obtained from the present study are given in Tables 1, 2, 3 and 4.

3.1 Effect of bioagents on plant growth parameters of rice

3.1.1 Effect on Plant height

The plant height of rice in various treatments differed significantly. At 30 days after transplanting, the plots treated with combination *P. fluorescens* + *T. harzianum* (27.16 cm) was recorded significantly higher plant height which was on par with Carbofuran (25.83 cm) and *T. harzianum* (25.43 cm) followed by *P. fluorescens* (23.83 cm) and *Bacillus subtilis* (23.03 cm) respectively. The similar trends were observed at 60, 90 and at the time harvest (Table.1, Fig.1)

3.1.2 Effect on Root length

The root length in various treatments differed significantly. All treatments registered higher length compared to check. The incorporation of consortium *P. fluorescens* + *T. harzianum* gave highest root length (21.27 cm) which was followed by carbofuran (17.63 cm), *T. harzianum* (17.50 cm) and *P. fluorescens* (17.23 cm) respectively. With respect to percent increase of root length over control, *P. fluorescens* + *T. harzianum* was significantly superior compared to rest of the treatments and recorded maximum root length (49.55 %) followed by carbofuran, *T. harzianum* and *P. fluorescens* respectively. With respect root weight combination of *P. fluorescens* + *T. harzianum* recorded highest fresh weight (6.84 g) and dry weight (3.75 g) followed carbofuran fresh weight (6.71 g) dry weight (3.63), *T. harzianum* fresh weight (6.69 g) dry weight (3.59 g), *P. fluorescens* fresh weight (6.51 g) dry weight (3.56 g), and *B. subtillis* fresh weight (6.22 g), dry weight (3.39 g), respectively. However, least root weight was observed in untreated control fresh weight (5.17 g) dry weight (2.42 g). (Table. 1)

3.1.3 Effect on grain yield and RKI

Data on the efficacy of bio-agents on grain yield and RKI of rice was recorded at the time harvests are presented in the Table. 2

All the treatments recorded significantly higher yield and least RKI compared to untreated control. The treatment application of *P. fluorescens* + *T. harzianum* was significantly superior compared to rest of the treatments and recorded maximum grain yield (45.60 q/ha) and least RKI (1.20) followed by carbofuran (44.13 q/ha) RKI (1.48), *T. harzianum* (43.80 q/ha) RKI (2.20), *P. fluorescens* (43.23 q/ha) RKI (2.56), and *B. subtillis* (42.57 q/ha) RKI (3.00) respectively (Table 2, Fig. 2).

In general, *P. fluorescens* + *T. harzianum*, carbofuran, *T. harzianum*, *P. fluorescens* and *Bacillus subtilis* were significantly superior recorded better plant growth parameters.

3.2 Effect on Nematode population in soil

The observation was recorded after the harvest of crop with respect to nematode population in soil revealed that the treatment combination of *P. fluorescens* + *T. harzianum* was significantly superior compared to rest of the treatments and recorded least nematode population (185.44) followed by carbofuran (215.22), *T. harzianum* (235.88), *P. fluorescens* (256.55) and *B. subtilis* (285.78) respectively. However, the

highest nematode population was observed in untreated control (681.22) (Table. 3).

All the tested bioagents were significantly superior over untreated control. Among the tested bioagents the least nematode population was recorded in contsorium of *P. fluorescens* + *T. harzianum*, carbofuran, *T. harzianum, P. fluorescens and B. subtilis* these were found to be effective for the management of rice root-knot nematode.

3.2.1 Effect on number of galls and egg masses

With respect to number of galls per root system and egg masses per galls *P. fluorescens* + *T. harzianum* showed least galls and egg masses 12.78 and 7.33 with reduction of 78.70% and 75.65% followed by carbofuran 15.67galls (73.88%), 9.33 egg mass (69.01%) and *T. harzianum* and 16.00 galls (73.33%) 12.00 egg mass (60.14%) respectively (Table.4 and figure 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. Combination of *P. fluorescens* + *T. harzianum* @20g/m^2 proved to be most effective treatment for the control of rice root-knot nematode followed by carbofuran, *T. harzianum, P. fluorescens* and *B. subtilis.* The present results are in line with the findings of [16] who found that the effect of *Pseudomonas fluorescens, Trichoderma viride* and carbofuran 3G independently and in combinations, significantly improves the plant growth in okra infested by *Meloidogyne incognita*. Similarly, [17] reported that combined application of *P. chlamydosporia, P. fluorescens, T. viride* and carbofuran resulted in significantly higher potato plant growth and lower cyst nematode population in soil and root. [18] Priya reported that *Trichoderma viride* had given lowest nematode population (209.28) least galling (12.80), gall index (1) and higher yield (3260 Kg /ha) followed by *Pseudomonas fluorescens* and *Bacillus subtillis.* [19] reported maximum mortality (>96%) of *M. graminicola* juveniles when exposed to culture filtrates (100 and 50% conc.) of *T. harzianum* Rifai. [20] Pathak and Kumar reported *T. virens* was more efeective in suppression of nematode population. The present results were also in consonance with the findings of [21] who reported that the soil application and root dip of *P. fluorescens or T. harzianum* + Carbofuran was found most effective in increasing yield of rice and suppressed the gall formation, egg mass production and soil population of *M. graminicola*. Application of Carbofuran to soil in nursery and main field at the rate of 1kg a.i. /ha reduced *M. graminicola* by over 90 per cent and resulted in increased yield of about 100%. [22]. Further, application of *P. flourescens* @ 20 g/m2 was found to be effective in reducing the nematode population and increasing the grain yields. [23] Anonymous showed that *Bacillus megaterium* significantly reduced the nematode galling [24, 25] who reported that induction of defense enzymes phenol, peroxidase (PO), polyphenol oxidase (PPO), phenyl ammonia lyase (PAL), super oxide dismutase (SOD) and chitinase by *P. fluorescens* isolate against rice root-knot nematode resulting in significant reduction in nematode infection. Integrated nematode management technology resulted in reducing the nematode population and also increased yields [26, 27] Sehgal *et al.* gave that application of carbofuran, *P. fluorescens* and *T. viride* to nursery bed reduces the galls increases yield. [28] who reported that combination of *P. fluorescens* at 20g/m² + cabrofuran (0.3 g a.i/m^2) maximum plant growth and grain yield with least nematode population followed by *T. viride* at 20g/m² + carbofuran. The reason for increase in growth parameters is due to that, the synergistic effect of *T. harzianum* on the production of nematicidal compounds critical in biocontrol may improve the efficacy of biocontrol bacteria against plant-parasitic nematodes. Considering the inconsistent performance of the biocontrol agents under field conditions, application of a mixture of compatible *T. harzianum* and *P. fluorescens* would more closely mimic the natural situation and might broaden the spectrum of biocontrol activity with enhanced efficacy and reliability of control. They also acts as growth promoting organism as they enhance the growth of plants height, root length and yield by reducing nematode population and serves as nematophagus fungus by producing some special structure, which kills the eggs and juvenile by producing toxins and alkaloids which hinders the growth and activity of nematodes [29]. Nematicides are not easily available, costlier, phytotoxic, health hazardous and cause much damage to the environment. They form a small proportion of total pesticides and herbicide usage. However, some compounds have been withdrawn from the market because of health hazards to production workers because of their detection at unacceptable levels in ground water. Unless, more acceptable nematicides are produced, the strategies for nematode management will be forced to change. The other methods of nematode management *viz*., crop rotation, field sanitation, fallowing, flooding and resistant crop varieties are having their own limitations and majority of the times not practicable. Nowadays, there is dearth of nematicides in Indian market As an alternative to nematicides of chemical origin many natural enemies attack plant parasitic nematodes in soil and reduced their population.

Table 1: Influence of bio-agents on plant growth parameters of rice infested by *M. graminicola*

Treatments	Plant height(cm)				Root length (cm)		Root weight	
	30 DAT	60 DAT	90 DAT	At harvest		% Increase over control	Fresh weight (gm)	Dry weight (gm)
T1= *Paceilomyces lilacinus* at 20 g/m²	20.56(4.59)*	40.60 (6.41)	62.13 (7.91)	75.77 (8.73)	15.07 (3.95)*	28.79	6.06 (2.56)	3.20 (1.92)
T_2= *Pseudomonas fluorescens* at 20 g/m²	23.83 (4.93)	44.20 (6.68)	65.50 (8.12)	78.40 (8.88)	17.23 (4.21)	37.72	6.51 (2.65)	3.56 (2.01)
T_3 = *Pochonia chlamydosporia* at 20 g/m²	21.46 (4.69)	42.53 (6.56)	63.93 (8.03)	76.60 (8.78)	16.60 (4.14)	35.36	6.08 (2.56)	3.37 (1.97)
T_4 = *Trichoderma harzianum* at 20 g/m²	25.43 (5.09)	44.67 (6.72)	65.97 (8.15)	80.27 (8.99)	17.50 (4.24)	38.68	6.69 (2.68)	3.59 (2.02)
T_5 = *Bacillus subtilis* at 20 g/m²	23.03 (4.85)	43.57 (6.64)	65.10 (8.10)	77.77 (8.84)	17.10 (4.20)	37.25	6.22 (2.59)	3.39 (1.97)
T_6 = *P. fluorescens* at 20 g/m² + *T. harzianum* at 20 g/m²	27.16 (5.25)	45.60 (6.79)	68.97 (8.33)	83.33 (9.15)	21.27 (4.66)	49.55	6.84 (2.71)	3.75 (2.05)
T_7 =Carbofuran 3G @ 0.3 ai /m^2	25.83 (5.13)	45.47 (6.78)	66.57 (8.19)	81.00 (9.04)	17.63 (4.26)	39.13	6.71 (2.68)	3.63 (2.03)
T_8 = Untreated control	16.13 (4.08)	35.57 (6.00)	56.63 (7.56)	65.87 (8.15)	10.73 (3.35)	-	5.17 (2.38)	2.42 (1.71)
S.Em ±	0.73	0.83	2.10	2.27	0.87	-	0.25	0.23
CD (P=0.05)	2.23	2.52	6.39	6.89	2.64		0.75	0.70

DAT= Days after transplanting

* Figures in the parenthesis are square root transformed value

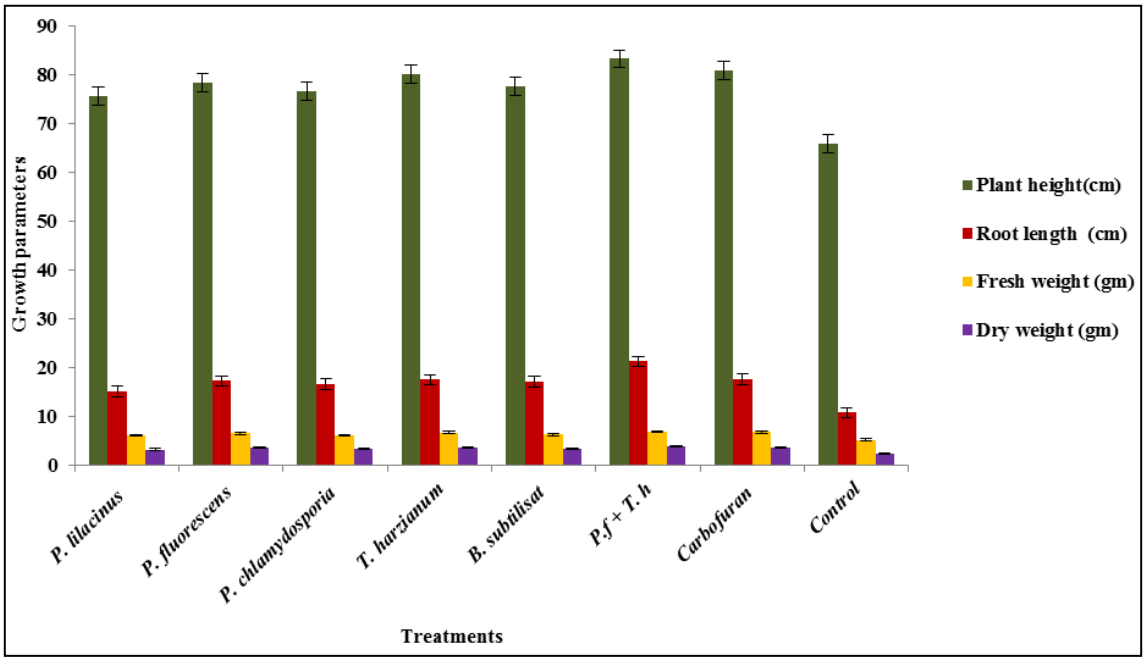

Fig 1: Effect of bioagents on plant growth parameters of rice infested by *M. graminicola*

Table 2: Effect of bioagents on yield and RKI of rice infested with *M. graminicola*

Treatments	Yield (Q / ha)	RKI (0-5)
T1= *Paceilomyces lilacinus* at 20 g/m²	39.40 (6.31)*	3.60
T_2= *Pseudomonas fluorescens* at 20 g/m²	43.23 (6.61)	2.56
T_3 = *Pochonia chlamydosporia* at 20 g/m²	41.40 (6.47)	3.42
T_4 = *Trichoderma harzianum* at 20 g/m²	43.80 (6.65)	2.20
T_5 = *Bacillus subtilis*at 20 g/m²	42.57 (6.56)	3.00
T_6 = *P. fluorescens* at 20 g/m² + *T. harzianum* at 20 g/m²	45.60 (6.79)	1.20
T_7 =Carbofuran 3G @ 0.3 ai /m^2	44.13 (6.68)	1.48
T_8 = Untreated control	36.33 (6.06)	4.63
S.Em.±	1.83	-
CD (P=0.05)	5.57	-

DAT= Days after transplanting
* figures in the parenthesis are square root transformed value

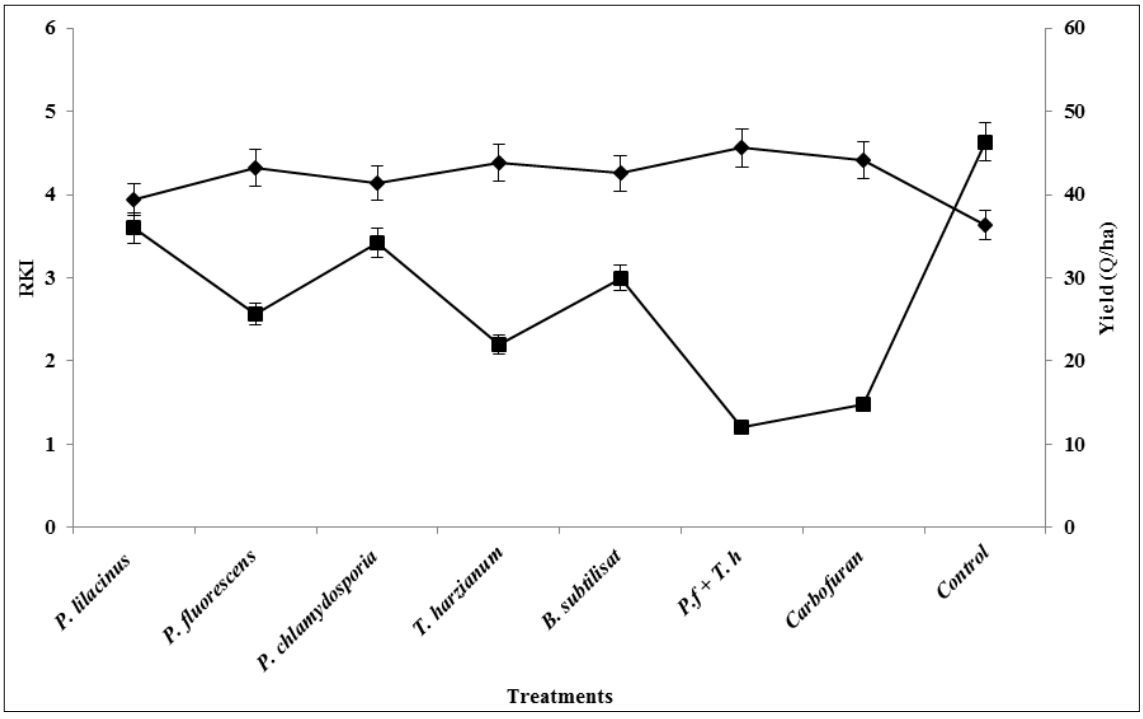

Fig 2: Effect of bioagents on grain yield of rice and Root Knot Index

Table 3: Effect of bioagents on nematode population of soil

Treatments	Nematode population / 200cc of soil				
	30 DAT	60 DAT	90 DAT	At harvest	% Decrease over control at harvest
T1= *Paceilomyces lilacinus* at 20 g/m²	470.00 (21.63)*	430.66 (20.71)	387.66 (19.65)	342.78 (18.46)	49.68
T_2= *Pseudomonas fluorescens* at 20 g/m²	426.00 (20.65)	365.22 (19.11)	316.33 (17.78)	256.55 (16.00)	62.33
T_3 = *Pochonia chlamydosporia* at 20 g/m²	457.67 (21.39)	413.22 (20.33)	364.11 (19.08)	316.22 (17.78)	53.58
T_4 = *Trichoderma harzianum* at 20 g/m²	401.00 (20.04)	337.67 (18.37)	284.00 (16.85)	235.88 (15.37)	65.37
T_5 = *Bacillus subtilis* at 20 g/m²	432.33 (20.80)	374.89 (19.36)	334.00 (18.27)	285.78 (16.89)	58.04
T_6 = *P. fluorescens* at 20 g/m² + *T. harzianum* at 20 g/m²	373.67 (19.33)	287.89 (16.98)	235.11 (15.35)	185.44 (13.63)	72.77
T_7 =Carbofuran 3G @ 0.3 ai /m²	391.00 (19.77)	327.22 (18.09)	272.77 (16.51)	215.22 (14.67)	68.40
T_8 = Untreated control	495.67 (22.26)	535.55 (23.15)	599.77 (24.50)	681.22 (26.09)	-
S.Em.±	20.71	23.90	22.42	24.56	-
CD (P=0.05)	68.22	72.50	68.32	74.53	-

DAT= Days after transplanting;
Average INP=550J_2 / Plot
* Figures in the parenthesis are square root transformed value

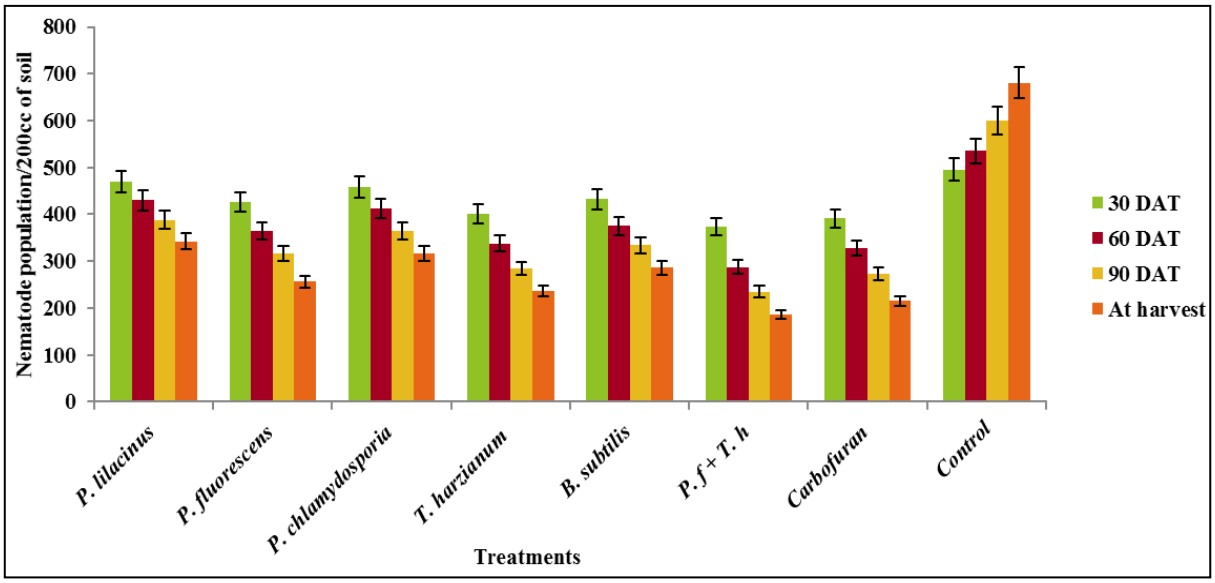

Fig 3: Effect of bioagents on nematode population of soil

Table 4: Effect of bioagents on reproduction of M. graminicola

Treatments	No. of galls per root system	% Decrease Over Control	No. of egg masses/ root system	% Decrease Over Control
T1= Paceilomyces lilacinus at 20 g/m²	44.66 (6.71)*	25.56	19.11 (4.42)	36.53
T_2= Pseudomonas fluorescens at 20 g/m²	24.44 (4.99)	59.26	14.11 (3.82)	53.13
T_3 = Pochonia chlamydosporia at 20 g/m²	42.33 (6.54)	29.45	17.22 (4.21)	42.80
T_4 = Trichoderma harzianum at 20 g/m²	16.00 (4.06)	73.33	12.00 (3.52)	60.14
T_5 = Bacillus subtilisat 20 g/m²	34.00 (5.85)	43.33	16.66 (4.13)	44.66
T_6 = Pseudomonas fluorescens at 20 g/m² + Trichoderma harzianum at 20 g/m²	12.78 (3.64)	78.70	7.33 (2.80)	75.65
T_7 =Carbofuran 3G @ 0.3 ai /m²	15.67 (4.02)	73.88	9.33 (3.13)	69.01
T_8 = Untreated control	60.00 (7.77)	-	30.11 (5.52)	-
S.Em. ±	2.08	-	1.58	-
CD (P=0.05)	6.31	-	4.79	-

DAT= Days after transplanting
* Figures in the parenthesis are square root transformed value

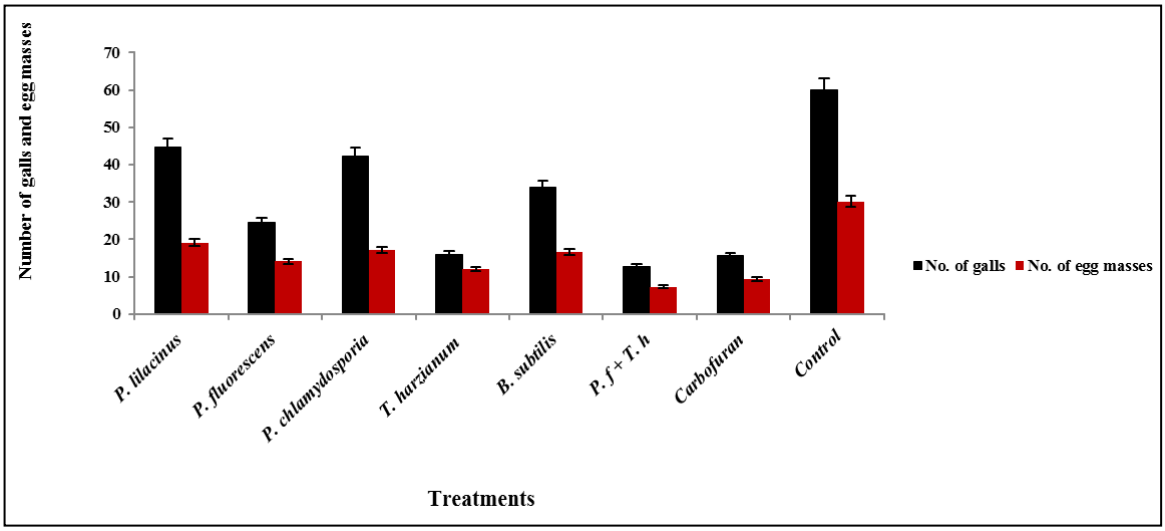

Fig 4

4. Conclusion

The present study implies that treatment combination of *P. fluorescens* + *T. harzianum* was found to be the best treatment as it recorded higher yield with least disease incidence it is may be due to compatible nature and combined effect.*T. harzianum* and *P. fluorescens* would more closely mimic the natural situation and might broaden the spectrum of biocontrol activity with enhanced efficacy and reliability of control. They also acts as growth promoting organism as they enhance the growth of plants height, root length and yield by reducing nematode population and serves as nematophagus fungus by producing some special structure, which kills the eggs and juvenile by producing toxins and alkaloids which hinders the growth and activity of nematodes.

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