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Evaluation of Bioformulations for Management of Root Knot Nematode (*Meloidogyne incognita*) Infecting Tuberose

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ABSTRACT

Field experiments conducted for testing field efficacy of bioformulations for management of root knot nematode (*Meloidogyne incognita*) infecting tuberose (*Polianthes tuberosa* L.) cultivar ´Calcutta single' in West Bengal. Eight treatments evaluated for managing root knot nematode problem in tuberose and results indicated that *Paecilomyces lilacinus*, *Trichoderma harzianum*, neem cake, *Pseudomonas fluorescens*, *Pochonia chlamydosporia* were effective and economic for managing *M. incognita* in tuberose. Among the bioformulations, application of *P. lilacinus* @ 5kg enriched with FYM 5t/ha was found to be the most effective and economic for reduction of root knot nematode infestation, nematode population and enhancement of cut-flower yield of tuberose. The application of *T. harzianum* @ 5kg with FYM 5t/ha was also comparable to carbofuran @ 1 kg a.i./ha for root knot nematode management in tuberose. Further, the present study demonstrated efficacy of single application of bioformulations against *M. incognita* infecting tuberose

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MRK conceived the project, identified the nematodes and supervised the study. TS conducted field experiments analyzed the data. MRK and TS wrote the article.

Key words:
Tuberose, bioformulation, neem, cut- flower yield, gall index, *Meloidogyne incognita*.

INTRODUCTION

Tuberose (*Polianthes tuberosa* L.) is commercially cultivated in open-field conditions for its fragrant flowers; loose flowers used as garlands, floral decorations, and spikes as cut-flowers. Root-knot nematode (*Meloidogyne incognita*) has been reported as one of the important limiting factors affecting commercial cultivation of tuberose (Sunderbabu and Vadivelu, 1988); reducer of flower yield up to 10% (Khan and Parvatha Reddy, 1992). The infection of root knot nematode makes the plants highly susceptible to attack of *Fusarium oxysporum* f.sp. *dianthi* (Rao *et al*., 2003). Infestation of nematode symptoms is characteristics; affected crop shows chlorotic foliage, general stunting, below ground portion with heavy root galling (Johnson, 1970). Root knot nematode problems in tuberose are widespread; majority of the tuberose fields in North and South India (Rao *et al*., 2001) are heavily damaged by the nematode. 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 agroecosystem depleted soil biota and withdrawal soil antagonists and beneficial organisms in soil environment promoted harmful plant pathogens including phytoparasitic nematodes. Root knot nematode is a common problem in almost all the crops grown in West Bengal, growing tuberose in the production system poses potential threat to the growers at higher density of soil nematode population. The present investigation was designed in a view to evaluate bioformulations for management of root knot nematode (*M. incognita*) in tuberose.

MATERIALS AND METHODS

Experimental site

Experiments were carried in the 2011 and 2012 growing season of tuberose (*cv* Calcutta single) in a field naturally infested with root knot nematode, *M. incognita* at the Central Research Farm of Bidhan Chandra Krishi Viswavidyalaya (BCKV), Gayespur (GPS: 23° 16' 53.5512" N/88° 21' 37.2888" E 23^0N, 9.75 m from MSL), West Bengal (India). The field is located in the New Alluvial Region of West Bengal and soil is typical sandy loam with poor water holding capacity. The total rainfall in the year of 2011 and 2012 was 2068mm and 1214mm, respectively. The maximum and minimum temperature during the crop growing season varied between 20 to 26°C and 33 to 37°C, respectively.

Field experiment

Experiment on efficacy of fungal formulations and neem based products for management of root knot nematode (*M. incognita*) in tuberose was carried out and the actual observations were taken from August 2011 to April 2012. The experimental field was divided into twenty-four plots, measuring 1.8m × 1.2m. All the

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treatments were arranged in randomized block design (RBD) with three replications. Each plot was transplanted with 24 infested bulbs of tuberose (cv. Calcutta single). The date of transplanting was on 24th March 2011 and spacing between the plants was 30cm × 30cm. Agronomic practices were adopted as per recommendations for the cultivar in the region. The observations on nematode population (J2) per 5g root, nematode population (J2) per 200cm^3 soil, stalk length (cm), rachis length (cm), number of florets (cm), stalk weight (g) yield (loose flower, kg/ha) were recorded during the growing period of the crop. Ten flower stalks randomly selected from each plot for above ground observations and three samples (soil along with root) were collected for assessment of root knot nematode infestation. Incremental cost benefit ratio (ICBR) for the treatments were calculated on the basis of prevailing costs (in INR) of components during the period of experimentation. Infestation of root knot nematode in the form of root galling in tuberose root was recorded on a 1-5 scale (1= no galls, 2=1-10 galls, 3=11-30 galls, 4=31-100 galls, 5= >100 galls per root system). Nematodes were extracted from 200cm^3 composite soil samples by Cobb's decanting sieving technique (Cobb, 1918) followed by modified Baermann's technique (Whitehead and Hemming, 1965). Root knot nematode population (J_2) from infested roots was recovered by the later method. Nematodes in the water suspension was killed and fixed (3.0% formaldehyde) for estimation of population; numbers of *M. incognita* was counted with the help of multi-chambered counting disk under stereoscopic binocular microscope (Zeiss, Stemi-2000C).

The details of the treatments tested were: T_1- Neem cake @ 1.0 t/ha, T_2- *Paecilomyces lilacinus* @ 5kg enriched with FYM 5t/ha, T_3: *Trichoderma harzianum* @ 5kg enriched with FYM 5t/ha, T_4- *Pseudomonas fluorescens* @ 5kg enriched with FYM 5t/ha, T_5- *Pochonia chlamydosporia* @ 5kg enriched with FYM 5t/ha, T_6- Carbofuran (Furadan 3G) @ 1 kg a.i./ha (as standard check), T_7- Neem seed powder @ 5 g/plant, and T_8 - Untreated control. Neem cake was applied in soil 15 days before planting of bulbs, fungal formulations, neem seed powder and carbofuran at planting. Formulations used in this study were procured from reliable organizations: *P. lilacinus* and *Pochonia chlamydosporia* from ICAR-National Bureau of Agriculturally Important Insects (ICAR-NBAII, Bangalore), *Trichoderma harzianum* from ICAR-Indian Institute of Horticultural Research (ICAR-IIHR, Bangalore), *Pseudomonas fluorescens* from Tamil Nadu Agriculture University (TNAU, Coimbatore), neem cake from Pest Control India Ltd, neem powder from Indiamart, and carbofuran (Furadan 3G) from FMC Rallis India Ltd. All the bioformulations contained minimum 2x10^6 cfu/g of talc based formulation.

Statistical analysis

All data were statistically analysed using Mstat-C software for analysis of variance (ANOVA) and treatment differences determined based on critical difference (CD) at P=0.05 level of significance.

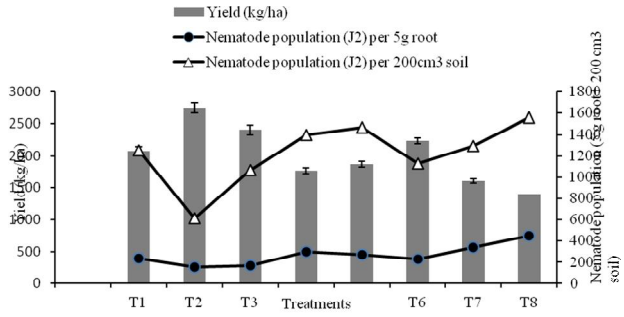

Fig. 1. Effect of treatments on cut-flower yield and on root knot nematode population (J2) infesting tuberose (2011)

RESULTS

The experiment was carried out with eight treatments during 2011-12. The adoption of treatments proved efficacy against *M. incognita* infesting tuberose and showed superiority over untreated control (Table I, Fig. 1). Among the treatments, T2 (*P. lilacinus* @ 5kg mixed with FYM 5t/ha) was found to be effective in reducing (67%) nematode (J2) population (150 J2 per 5g root) and root galling (gall index ~2.00). This was at par with T3 (*T. harzianum* @ 5kg mixed with FYM 5t/ha) where nematode population was recorded 168 (63%) per 5g root with almost similar level of root galling (gall index ~2.07). The plots receiving T6 (carbofuran @ 1 kg a.i./ha) had greater nematode population (228J2/5g root, 49%) with mean gall index. nearly 2.11. Similarly neem cake @ 1.0 t/ha (T_1) treated plots had relatively greater nematode population about 238 J2 per 5g root, 47% higher than untreated control, and root galling (GI~2.22). Relatively high nematode population (J2) per 5g root and root galling were recorded in T_4 (300; 33% and GI~2.33) and T_7 (336; 25% and GI~2.67). The experimental field was not free from other plant parasitic nematodes (PPNs). Therefore, occurrence of other PPNs was also encountered and their population were also estimated per 200cm^3 +5g soil at the harvest of the crop, presented in Table I. The populations of *M. incognita (*253 per 200cm^3

Table I.- Efficacy of bioagents and neem products for management of *Meloidogyne incognita* infesting tuberose during 2011.

Treatments*	Gall index (1-5)	Stalk length (cm)± SD	Inflorescence length (cm) ± SD	Total no. of florets ± SD	Nematode population (J_2) per 5g root	Nematode population (J_2) per 200cm^3 soil	Stalk weight (gm)	Yield of cut-flower (kg/ha)	ICBR
T1	2.22	63.50±2.98 (60.75-66.67)	24.62±2.82 (22.17-27.70)	22.69±1.50 (21.00-23.83)	238	157.33 (128-178)	35.00±8.89 (28-45)	2080.94	1:1.78
T2	2.00	66.08±4.81 (60.72-70.03)	27.26±1.73 (26.00-29.23)	25.48±1.57 (24.17-27.22)	150	102.50 (86-126)	38.33±5.77 (35-45)	2742.28	1:21.40
T3	2.07	65.26±3.94 (60.75-68.00)	26.04±2.42 (23.83-28.63)	24.30±2.27 (21.96-26.50)	168	118.00 (96-148)	36.00±6.56 (30-43)	2406.37	1:18.00
T4	2.33	62.28±0.63 (61.56-62.64)	23.55±3.99 (19.67-27.64)	21.21±1.04 (20.17-22.25)	300	222.67 (184-248)	27.33±2.52 (25-30)	1766.13	1:6.41
T5	2.33	63.37±3.75 (59.72-67.22)	24.65±3.47 (21.08-28.02)	21.75±2.27 (19.46-24.00)	270	195.40 (176-226)	33.33±2.89 (30-35)	1868.06	1:7.72
T6	2.11	64.69±2.24 (63.33-67.28)	25.99±2.70 (23.00-28.25)	22.25±1.08 (21.17-23.33)	228	134.67 (116-168)	35.00±5.00 (30-40)	2239.19	1:14.83
T7	2.67	60.93±3.59 (56.78-63.06)	24.96±0.98 (24.17-26.06)	21.69±0.35 (21.42-22.08)	336	206.00 (180-224)	30.00±5.29 (26-36)	1606.40	1:0.13
T8	3.00	59.56±1.11 (58.28-60.22)	21.17±1.53 (19.50-22.50)	19.04±1.15 (18.00-20.28)	450	244.83 (220-284)	27.33±2.52 (25-30)	1405.09	-
SEM±	-	1.22	1.06	0.74	-	-	-	59.12	-
CD(P=0.05)	-	3.69	3.20	2.24	-	-	-	179.32	-

*T_1, Neem cake @ 1.0 t/ha; T_2, *P. lilacinus* @ 5kg mixed with FYM 5t/ha; T_3, *T. harzianum* @ 5kg mixed with FYM 5t/ha; T_4, *Ps. fluorescens* @ 5kg mixed with FYM 5t/ha; T_5, *Pochonia chlamydosporia* @ 5kg mixed with FYM 5t/ha; T_6, Carbofuran @ 1 kg a.i./ha; T_7, Neem seed powder @ 5 g/ plant; T_8, Untreated control; Tuberose variety: Calcutta single.

Table II. Efficacy of bioagents and neem products for management of *Meloidogyne incognita* in tuberose during 2012.

Treatments*	Gall index (1-5)	Stalk length (cm)± SD	Inflorescence length (cm) ± SD	Total no. of florets ± SD	Nematode population (J_2) per 5g root	Nematode population (J_2) per 200cm^3 soil	Stalk weight (gm)	Yield of cut-flower (kg/ha)	ICBR
T1	2.67	63.67± 1.53 (62.00-65.00)	23.50±3.04 (21.50-27.00)	23.17±4.65 (20.00-28.50)	300	232.33 (205-252)	35.33±4.16 (32-40)	4040.82	1:10.67
T2	2.07	68.33±4.73 (63.00-72.00)	27.67±2.52 (25.00-30.00)	29.00±4.58 (25.00-34.00)	180	161.67 (145-180)	41.00±3.61 (37-44)	4854.94	1:78.88
T3	2.11	66.00±3.61 (62.00-69.00)	25.33±4.62 (20.00-28.00)	27.17±3.69 (23.00-30.00)	205	210.00 (194-230)	39.67±2.52 (37-42)	4566.87	1:82.10
T4	2.67	61.67±3.51 (58.00-65.00)	22.83±4.37 (18.00-26.50)	25.00±7.00 (18.00-32.00)	336	276.00 (246-310)	34.33±3.79 (30-37)	3540.12	1:62.80
T5	3.00	62.00±2.65 (59.00-64)	23.33±2.08 (21.00-25.00)	22.11±3.56 (19.00-26.00)	386	255.00 (210-290)	34.78±2.91 (32-38)	3766.13	1:62.77
T6	2.33	64.67±3.21 (61.00-67.00)	24.17±4.01 (20.00-28.00)	24.28±1.85 (22.33-26.00)	240	246.67 (220-284)	37.50±3.04 (34-39)	4287.81	1:76.23
T7	3.00	62.83±7.01 (55.00-68.50)	21.50±4.77 (16.50-26.00)	21.33±2.52 (19.00-24.00)	440	288.67 (256-330)	33.00±2.64 (30-35)	3300.85	1:2.09
T8	3.33	57.67±2.52 (55.00-60.00)	19.00±2.08 (18.00-22.00)	21.28±6.10 (14.50-26.33)	536	318.67 (286-360)	31.67±4.04 (28-36)	2516.20	-
SEM±	-	2.29	1.93	2.68	-	-	-	166.22	-
CD(P=0.05)	-	6.94	5.87	8.12	-	-	-	504.19	-

For details of treatment groups, see Table I.

+5g soil) and *R. reniformis* (464 per 200cm^3 soil) were found relatively lesser abundance in T_2 in comparison to T3 (*M. incognita* ~286 and *R. reniformis* ~624), T_6 (*M. incognita*~363 and *R. reniformis*~710) and T_1 (*M. incognita*~395, *R. reniformis*~630). The stalk length (66.08±4.81), inflorescence length (27cm) and total number of florets (25) were also more in T_2 and T_3 (65cm, 26cm and 24). The influence of nematode infection probably caused reduction in stalk length, inflorescence length (cm) and total number of florets in T_7 (61, 25 and 22) and T_4 (62, 24 and 21). The cut-flower yield (Fig. 1, Table I) and stalk weight were recorded highest in T_2 (2742 kg/ha, 38g) followed by T_3 (2407 kg/ha, 36g) T_6 (2239, 35g) and T_1 (2081, 35g). While relatively low cut-flower yield (kg/ha) and stalk weight (g) were found in T_7 (1606, 30) and T_4 (1766, 27). ICBRs calculated for T_2, T_3, T_6, and T_5 were 1:21, 1:18, 1:15, and 1:8, respectively.

The same trial was conducted during 2012 and results are presented in Table II. Lowest (gall index ~ 2.07) root gall index and nematode (J2) population (180 per 5g root) was recorded in T2 (*P. lilacinus* @ 5kg mixed with FYM 5t/ha) and followed by T3 (gall index ~2.11; 205 per 5g root). The treatments, T6 (carbofuran @ 1 kg a.i./ha) and T1 (Neem cake @ 1.0 t/ha) recorded relatively lesser nematode (Fig. 2) populations (240 per 5g root, 300 per 5 g root), and gall indices (~ 2.33, 2.67). While the maximum nematode population and root gall indices was observed in T4 (336; GI~2.67) and T7 (440; GI~3.00). Other PPNs predominantly root knot and reniform nematodes were also recorded per 200cm^3 +5g soil at the time of final harvesting (not shown in table). The population of *M. incognita* (342 per 200cm^3 +5g soil) and *R. reniformis* (529 per 200cm^3 soil) were found less in T_2 in comparison to T3 (*M. incognita*~415; *R. reniformis* ~624), T_6 (*M. incognita*~487; *R. reniformis*~550) and T_1 (*M. incognita* ~532; *R. reniformis* ~574). Among the yield parameters like stalk length (68.33±4.73), inflorescence length (27.67±2.52) and total number of florets (29.00±4.58) were also recorded highest in T_2 and T3 (66cm, 25cm, 27cm). In addition, cut-flower yield (Table II, Fig. 2) and stalk weight were recorded highest in T_2 (4855 kg/ha, 41g) followed by T_3 (4567 kg/ha, 40g), T_6 (4229, 37g) and T_1 (4041, 35g). The lowest cut-flower yield (kg/ha) and stalk weight (g) were found in T_7 (3300, 33) and T_4 (3540, 34). ICBRs calculated for T_3, T_2, and T_6, were 1:82, 1:79, and 1:76, respectively (Table II). Considering effectiveness and economics of the treatments, *P. lilacinus* @ 5kg mixed with FYM 5t/ha (T_2) could be recommended for management of root knot nematode in tuberose. The effectiveness of *T. harzianum* @ 5kg mixed with FYM 5t/ha (T_3) was also found comparable with carbofuran @ 1 kg a.i./ha(T_6), therefore, this may also be considered for the purpose.

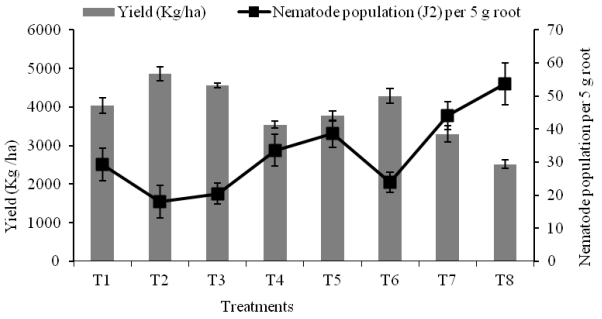

Fig. 2. Effect of treatments on cut-flower yield and on root knot nematode population (J2) infesting tuberose (2012).

DISCUSSION

The biocontrol potential of fungal bioagents particularly using *P. lilacinus* and *T. harzianum* either alone or in combination with neem cake and FYM against *M. incognita* infecting many crops have been tested and often more than one applications have been suggested (Rao, 2007) for assured nematode management. The present study proved effectiveness of bioproducts using single application in tuberose and results in respect of root knot nematode management were found comparable to carbofuran. Further studies on *P. lilacinus* alone and in combination with neem leaf extract (5%) both as bulb and soil drench treatment increased plant growth, flower yield of tuberose and reduction of root galling (Nagesh *et al.*, 2007). Our findings are in conformity with observation of Chawla and Sellaperumal (2010); slurry coating of *P. lilacinus* (8% w/w) in infected tuberose bulb effectively managed *M. incognita* with maximum reduction of 60% in galls, 70% in egg masses per plant and 66% in soil population of nematode. The efficiency of *T. harzianum* proved comparable to that of carbofuran for the management of root knot nematode in tomato (Khan *et al.*, 2011). Among the chemicals tested earlier for managing root knot nematodes in tuberose, the nursery bed treatment with carbofuran or phorate at 60g/m^2 (Parvatha Reddy and Rao, 2001) and soil application of aldicarb at 20 mg/plant or carbofuran 3G at 10-20 g/plant were proved most effective. However, uses of agrochemicals at the higher doses for controlling root knot nematode are of major concerns for environmental and soil health; toxic effects denude soil macro and micro fauna, reduce activity of

antagonistic and predatory populations, and often reduced biocontrol efficacy of introduced biocontrol agents. The present study included treatments for testing bioproducts in comparison to carbofuran for the control of root knot nematodes. The growth promoting rhizobacteria (*Pseudomonas fluorescens*) suppressed infestation of *M. incognita* in tomato and improved fruit yield (Haq *et al*., 2011). Our results with the use of neem cake, *P. fluorescens* and *Pochonia chlamydosporia* also gave control of root knot nematodes and indicated profit for the practices. The relative performance of *P. lilacinus* and *T. harzianum* were more promising to reduce infestation of root knot nematode and enhancing cut-flower yield of tuberose. The results supported the reporting of Sultan *et al*. (2011) who obtained good control with application of carbofuran, and recorded increased bulb yield despite having significant root galling due to *M. incognita* in plots treated with neem cake, neem seed powder, *P. lilacinus* and *T. harzianum*. Therefore, the bioproducts tested here could be recommended for managing root nematodes on tuberose in organic production.

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