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Management of nematodes using liquid formulations of *Purpureocillium lilacinum* in tuberose

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Abstract
Plant parasitic nematodes are important biotic restrictions in the horticultural systems. Root-knot nematode, *Meloidogyne incognita* is a major economic pest in various crops. Wide usage of pesticides against parasitic nematode causes health hazards; hence there is an urgent need for an alternative. Biocontrol agents serve as an alternative. *P. lilacinum* is a nematophagous fungus which is used as a biocontrol agent. Tuberose (*Polianthes tuberosa* L.) is an important ornamental crop in India and it has great economical value as a cut flower and in the perfume industry. Root knot nematodes cause serious damage to roots of tuberose, resulting in 14-15% yield loss. Keeping this in view, the present studies were under taken to evaluate the efficacy of liquid formulation of *P. lilacinum* in tuberose. The experiments were conducted during 2015-2016 in both greenhouse and field conditions. Six treatments were used in this study. In greenhouse conditions, corms treated along with soil application of 2 tons/ha of vermicompost enriched with 5 l of *P. lilacinum* have showed a maximum increase in the plant growth parameters like spike length (71.25 cm), spike weight (44.34 g), root length (15.38 cm), root weight (7.18 g) and reduced nematode gall index (1.5). Field assessment confirmed significant inhibition of nematode reproduction, inhibition of root galling and an increase of tuberose yield compared with the control.

Keywords: *Purpureocillium lilacinum*, liquid formulations, bio efficacy studies, enrichment, tuberose

1. Introduction
Tuberose (*Polianthes tuberosa* L; Amaryllidaceae) is mainly cultivated for the production of flower spikes and loose flowers on a commercial scale for the domestic market. It is estimated that the production of loose flowers and cut flowers of tuberose is 0.0277 million tons and 156.07 million numbers, respectively [1]. They can be cultured in fields and under greenhouse conditions. The production of field grown cut flowers has become quite popular in recent years.

Root-knot nematodes (*Meloidogyne* spp.) cause serious threat to commercial cultivation of tuberose. Infestation of *M. incognita* was reported to be wide spread in almost all the tuberose growing regions of south India [2]. Crop infested by nematodes results in chlorotic foliage, general stunting and heavy root galling in corms/roots [3]. Although chemical nematicides have an instantaneous effect over the control of nematodes, their usage has been restricted due to environmental and health hazards.

Nematodes also predispose the roots to easy entry of other soil borne fungal pathogens and the resulting nematode disease complex is responsible for drastic reduction in yield and financial loss to the growers. Considering the hazardous effects of chemical pesticides on biotic life and the environment, the biological control alternatives are paid more attention by scientists and growers. Bio control agents (BCAs) are recommended throughout the world for management of several diseases [4].

According to Fiedler and Sosnowska [5], *Purpureocillium lilacinum* acts as an effective biocontrol agent to manage the root knot nematodes by producing secondary metabolites which control nematode population in soil [6].

Solid formulations suffer from major drawbacks such as shorter shelf life, high contamination and farmers are facing difficulties in using it, so we have developed liquid formulations.

The success of a BCA mainly depends on its proper deliver systems under field conditions. Earlier reports have proven that enrichment of BCA with neem cake or Farm Yard Manure has shown better antagonistic activity against nematodes [7, 8]. Hence this study was aimed to investigate the effect of liquid formulation of *P. lilacinum* against *M. incognita* under greenhouse and field conditions in Tuberose.

2. Materials and Methods

2.1 Maintenance of nematode culture

Pure culture of root knot nematode *M. incognita* was maintained in tomato under the greenhouse conditions at ICAR–Indian Institute of Horticultural Research (IIHR), Bengaluru. The identity of female *M. incognita* was confirmed based on their perineal cuticular pattern [9]. The nematode eggs were rinsed incubated and second stage juveniles (J_2) were collected daily for 5 days [10] and were observed using stereo zoom microscope (Motic, Hong-Kong). These nematodes (J_2) were counted per ml of suspension in three replicated aliquots and 1000 J_2 per pot were inoculated for all respective treatments.

2.2 Biocontrol agents

Biocontrol agent (BCA) *Purpureocillium lilacinum* (PL-IV) was isolated from soil samples from Shimoga, Karnataka. The microbe isolation was done by the serial dilution method. The isolated colonies were identified as *P. lilacinum* by morphological and molecular characteristics. Liquid formulations of BCAs were prepared as per the standard procedures of Rao [4] with suitable modifications. The population in terms of colony forming units (CFU) was maintained for *P. lilacinum* in formulations.

2.3 Root colonization

To evaluate the root colonization of *P. lilacinum*, the roots were washed; pieces of about 3-4 mm were used. One gram sample of roots were used and root colonization was assessed by serial dilution method.

2.4 Bio efficacy studies

Liquid formulations were prepared as per the procedures of Rao [4] with slight modifications; the formulations were prepared as aqueous suspension (1% A.S) and used under green house and field conditions. For all the treatments with bioagents, tuberose corms were treated initially with (PL 1% A.S) at 20 ml/kg corms. The corms without bioagent treatment were also sown according to the treatment schedule. Enrichment process was followed by Rao [11] the formulations were enriched with vermicompost, set aside under shade for 15 days with optimum moisture and temperature at 24-28°C was maintained for the proper enrichment of vermicompost with *P. lilacinum*.

2.5 Evaluation of liquid formulation of *P. lilacinum* against *M. incognita* under greenhouse condition

Efficacy of liquid formulation of *P. lilacinum* was tested against nematode in tuberose var. Nirantara under pot culture condition at Department of Nematology, ICAR-IIHR. Completely Randomized Design (CRD) was followed with five replications during August to December, 2015 and during June to October, 2016 by maintaining at ambient temperature range of 28 to 32^0C. Uniformed sized healthy corms of tuberose var. Nirantara were sown in 3 kg pots filled with a sterilized pot mixture (red soil: sand: FYM in the ratio 2:1:1) at one bulb/pot. There are six treatments.

Experiment was concluded after 150 days of application of the treatments and the plants were pulled out to assess the effect of liquid formulation against the growth and yield parameters of the plant and the reduction in nematode. Gall index was assessed by counting the number of galls per root system and rating was given based on their numbers (1= no galls; 2=1-25 % galls; 3=26-50% galls; 4=51-75 % galls; 5=76 -100 % galls per root system) [12], soil and root nematode populations [13] plant growth parameters like spike length, spike weight, root length, root weight and bulb weight were also recorded.

2.6 Evaluation of liquid formulation of *P. lilacinum* against nematode under field condition.

The experiment was conducted for two seasons in tuberose during April to August, 2015 and May to September, 2016 in pathogen sick plot in ICAR-Indian Institute of Horticultural Research, Bangalore, India (location 11.29^1N; 75.82^1E) to evaluate bio-efficacy of the liquid formulations. The liquid formulations were enriched with vermicompost. Vermicompost enriched with bio agents were applied to soil in the experimental plots before sowing. There are 6 treatments; T_1- *P. lilacinum*1% A.S at 20 ml/kg seed; T_2 -T_1+ 2 tons of vermicompost/ha enriched with 2. 5 l of *P. lilacinum*; T_3 - T_1+ soil application of 2 tons of vermicompost t/ha enriched with 5 l of *P. lilacinum*; T_4 - Soil application of vermicompost at 2 tons/ha; T_5 - Carbofuran at 1kg a.i./ha; T_6 - Control (Untreated). The formulations were applied at monthly intervals. The initial population of nematodes was estimated as 112±2.8 J_2 per 100 cc of soil for the first experiment and followed by 118±2.6 J_2 per 100 cc of soil. The plot size of the bed was 2m X 1.5m, for this experiment random block design (RBD) was followed with five replicates. The experiment was completed after 240 days and the following observations were recorded. *viz*., root length, spike length, root weight, spike weight, flower yield, gall index, soil nematode population, and no. of egg masses/g root were recorded.

2.7 Statistical analysis

The effect of different treatments on growth parameters, yield, gall index, nematode population and diseases index were tested using ANOVA and the critical difference were determined using Duncan's Multiple Range Test. As the results were similar in both experiments the data from two seasons were pooled and ANOVA was executed using SPSS ver.10.0 [14].

3. Results and discussion

3.1 Identification of *Meloidogyne* species associated with tuberose

Meloidogyne species were identified by the cuticular markings present in the perenial area of the matured female. The cuticular pattern in the perennial area was observed with high dorsal arch and flattened top. Based on the cuticular pattern, the species was identified as *M. incognita.*

3.2 Effect of liquid formulation of *P. lilacinum* against *M. incognita* under greenhouse conditions

The experiments conducted under greenhouse exhibited better results as compared with control. Among the treatments, T_3 where the enrichment of *Paecilomyces* was done with vermicompost @ 5 t/ha was found to be more effective in reducing Gall Index (Table.1) and increasing plant growth parameters like spike length (71.25cm), spike weight (44.34 g), root length (15.38 cm), root weight (7.18 g) and bulb weight (34.84 g), (Fig 1). Root colonization of *P. lilacinum* in soil ($5.2X10^{-4}$) and in root ($5.8X10^{-4}$) was recorded (Table.1).

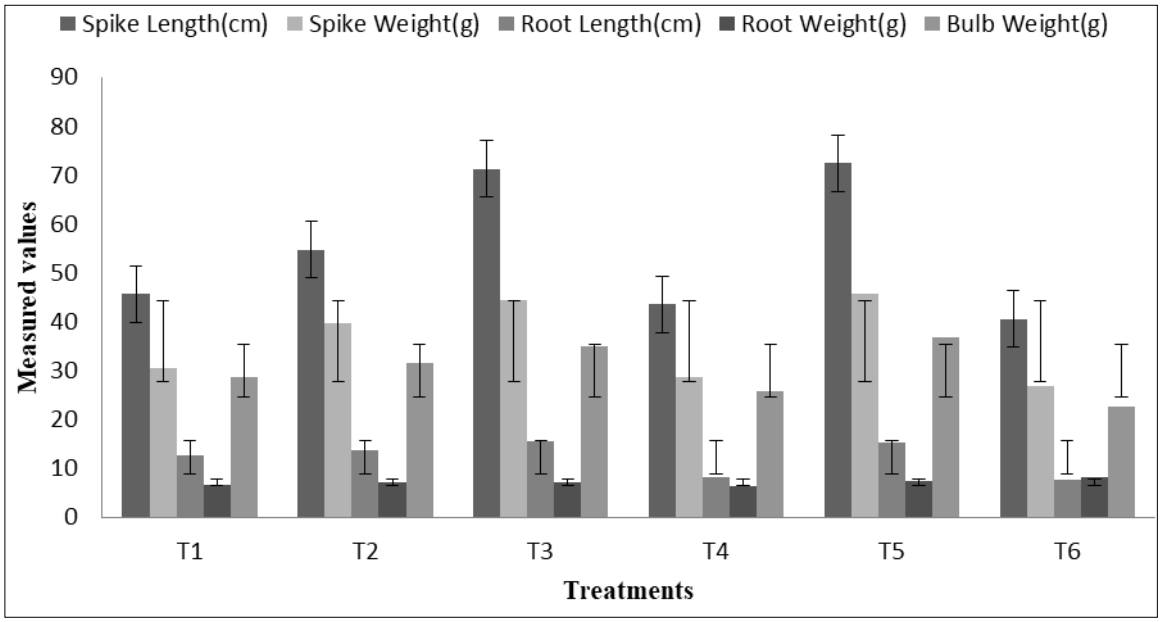

Fig 1: Effect of *P. lilacinum* liquid formulations on plant growth parameters under greenhouse conditions in tuberose

Table 1: Gall index and density of *P. lilacinum* in tuberose under greenhouse conditions

Treatments	Root gall index 1-5 Scale	Density of *P. lilacinum* (X10^{4}CFU/g)	
		Soil	Root
T_1	3.1±0.5	3.2	3.7
T_2	2.2±0.5	3.9	4.2
T_3	1.2±0.5	5.2	5.8
T_4	4.1±0.4	-	-
T_5	1.6 ±0.5	-	-
T_6	5.0 ±1.3	-	-
SED	0.24	0.25	0.26
C D (*P*=0.01)	0.67	0.77	0.80

T_1 – Seed treatment (ST) with *P. lilacinum* at 20 ml kg/ seed;
T_2– ST + soil application of 2 tons /ha of vermicompost enriched with 2.5 l of *P. lilacinum*;
T_3 – ST+ soil application of 2 tons /ha of vermicompost enriched with 5 l of *P. lilacinum*;
T_4 –Soil application of vermicompost alone at 2 tons /ha; T_5 – Carbofuran at 1 kg a.i/ ha; T_6 –Untreated control.

3.3 Effect of liquid formulation of *P. lilacinum* against in tuberose under field conditions

Field trials were conducted for two consecutive seasons in 2015-16. The treatments proved significantly effective against *M. incognita* infesting tuberose. Among the treatments, T_3 (PL@ 5 t/ha) was found to be the most effective in reducing nematode population in roots

23.42 females /g root and in soil 60.23Juveniles/100cc soil and lower gall index (1.5) and increasing stalk length (63.58 cm), inflorescence length (17.52 cm) total number of florets/spike (24.46) and stalk weight (41.15 g). In T_2 nematode densities in soil 92.34 and root 27.41, gall index (2.4) were recorded and in T_1 3.2gall index, nematode populations in 110.54juveniles/100 cc soil and 40.19 females/g root) were recorded. The highest flower yield was recorded in T_3 (26.4) t/ha, followed by T_2 (23.2), T_1 (22.8) respectively. Untreated control (T_6) recorded maximum nematode population (52.14females/g root); 138.46 juveniles/100cc soil) with the gall index of 5. Root colonization of *P. lilacinum* in soil ($4.5X10^{-4}$) and in root ($4.8X10^{-4}$) was recorded (Table. 2).

Rao [11] suggested that the fungal bioagents like *P. lilacinum* enriched in vermicompost and FYM were found useful to use against *M. incognita* for the successful management of nematodes. Maboeta [15], Mkhabela [16] have reported that vermicompost is very suitable for maintaining soil pH. The researchers believed that increasing the nutrients in the soil will increase nutrient uptake and plants can grow better and increase yield [17].

P. lilacinum is very promising bio agent to control the root-knot nematode *M. incognita* [18]. It has been very effective in controlling the population of nematodes under numerous conditions (Cannayane and Sivakumar [19], Anastasiadis) [20]. Khan and Saxena [21] report that *P. lilacinum* is competent in parasitizing nematode eggs, juveniles and females on various crops in the world. The soil treatment from the earlier stages reduced root galling and number of egg masses. *P. lilacinum* was effective against the root knot nematode which significantly reduced galls, egg masses and helped in the enhancements of plant growth [22-25]. Our results were in accordance with these reports. The in dissemination of nematode egg was mainly done by the serine protease enzyme [26-28]. According to Westphal [29] application of *P. lilacinum* prior to the transplantation of the crop has reduced the nematode population.

Oka [30] reported that usage of manures reduced the nematode population due to the nematicidal compounds which are released during decomposition, which in turn help in the multiplication of microorganisms Application of enriched biocontrol agents from the initial stages of the crop and the soil application were reported to be the best management practice in integrated nematode management [31, 11].

Table 2: Bio-efficacy of liquid formulations of *P. lilacinum* in tuberose under field conditions

Treatments	Gall Index	Stalk length (cm)	Inflorescence length(cm)	Total no. of florets	Nematode population		Stalk weight(g)	Yield of cut flowers (t/ha)	Density of *P. lilacinum*(X10^{4}CFU/g)	
					Soil/100cc	Root /g			Soil	Root
T_1	3.2	45.62	9.68	15.74	110.54	40.19	30.56	22.8	3.2	3.4
T_2	2.4	54.68	12.58	18.64	92.34	27.41	36.57	23.2	3.7	3.9
T_3	1.5	63.58	17.52	24.46	60.23	23.42	41.15	26.4	4.5	4.8
T_4	4.1	43.58	8.52	14.32	128.75	48.37	28.67	22.4	-	-
T_5	1.9	64.21	16.58	23.68	65.22	25.66	42.58	26.7	-	-
T_6	5	40.48	5.28	12.69	138.46	52.14	26.74	22.2	-	-
SEd	0.15	1.92	1.30	2.04	2.24	1.39	2.23	1.04	0.13	0.08
C D (*P*=0.05)	0.33	4.01	2.73	4.27	4.68	2.91	4.66	2.17	0.31	0.20

T_1 – Seed treatment (ST) with *P. lilacinum* at 20 ml kg/ seed; T_2– ST + soil application of 2 tons /ha of vermicompost enriched with 2.5 l of *P. lilacinum*; T_3 – ST+ soil application of 2 tons /ha of vermicompost enriched with 5 l of *P. lilacinum*; T_4 –Soil application of vermicompost alone at 2 tons /ha; T_5 – Carbofuran at 1 kg a.i/ ha; T_6 –Untreated control.

4. Conclusion

P. lilacinum has proved to be a successful bioagent in the management practices of *M. incognita* and hence it can be used as an effective biocontrol agent in various crops. The liquid formulation of *P. lilacinum* 1% A.S. was found to be effective when it was enriched in 2 tons vermicompost with 5 l of *P. lilacinum* and applied through the soil and drenching methods in tuberose exhibited higher antagonism towards *M. incognita* and enhanced tuberose plant growth parameters and yield. Our bioefficacy studies have also suggested that the continuous usage of enriched biopesticides helps in increasing the crop yield and reduces the nematode infestation. Hence, this bio-agent can be used as an effective component in integrated management systems for the effective management of root-knot nematode in tuberose in a sustainable manner.

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