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Management of root-knot nematodes (*Meloidogyne* spp.) using different bio-agents under field conditions

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Abstract
A field experiment conducted to study the efficacy of different bio-agents for the management of root-knot nematodes, *Meloidogyne* spp. in okra indicated that FYM @ 0.2% (w/w) enriched with *Purpureocillium lilacinum* (2 ×10^6cfu/g) followed by *Pseudomonas fluorescens* (2 × 10^6cfu/g) was found effective to manage root-knot nematodes and increased okra fruit yield by 76.05% and 70.32% respectively over control.

Keywords: Management, bio-agent, okra, *Meloidogyne* spp., FYM, *Purpureocillium lilacinum*, *Pseudomonas fluorescens*

Introduction
Okra (*Abelmoschus esculentus* L. Moench) is an important vegetable crop belonging to the family Malvaceae and grown throughout the tropical and subtropical regions of the world. In world, India ranks second in production of vegetables, next to China. In India, okra is cultivated in 0.53 million ha area with an annual production of 6.35 million tonnes with productivity of 12 tonnes/ha. In India, it is widely cultivated in Bihar, Orissa, West Bengal, Andhra Pradesh, Madhya Pradesh, Karnataka, Gujarat and Assam. Okra is one of the important vegetable crops of Gujarat, covering 65.99 thousand ha area with an annual production of 759.04 thousand MT and productivity of 11 MT/ha (Anon., 2014). Ahmedabad, Anand, Gandhinagar, Dang, Narmada, Navsari, Panchmahal, Bharuch, Sabarkantha, Surat and Tapi are major okra growing districts in Gujarat state. Various researchers also estimated yield losses ranging from 13.51 to 27.0% (Sikora and Fernandez, 2005 and Anon., 2015). In Gujarat, 39.74% loss in okra yield due to root-knot nematodes has been reported (Anon., 2015). The root-knot nematode is sedentary endoparasite which produces disease symptoms both on above and below ground plant parts. It includes severe root galling, stunted growth, reduced fruit size and yield, chlorotic leaves and in some cases wilting in association with other soil born pathogens. This dreadful pest poses a serious problem because of its high reproductive potential and rapid turnover of generations. Therefore, present investigation was carried out to manage root-knot disease in okra field.

Materials and Methods
The present investigation was carried out at Department of Nematology, B. A. College of Agriculture, Anand Agricultural University, Anand during 2016-17.There were total seven treatments and four replication in randomized block design. (T_1: *Purpureocillium lilacinum* (2 × 10^6cfu /g) @ 5 kg/ha, T_2:*Pochonia chlamydosporia* (2 × 10^6cfu /g) @ 5 kg/ha, T_3: *Pseudomona fluorescens* (2 × 10^6cfu /g) @ 5 kg/ha, T_4: *Trichoderma viride* (2 × 10^6cfu /g) @ 5 kg/ha, T_5: *Trichoderma harzianum* (2 × 10^6cfu /g) @ 5 kg/ha,T_6: FYM @ 2.5 t/ha and T_7: Control (Untreated check).In field, 3.0 × 5.1 m sized 28 beds were prepared.For enrichment of FYM, each bio-agent was mixed with FYM @ 0.2% (w/w) and this mixture was kept moist for 15 days.Soil sample was collected from the field area and processed by Petridish Assembly Method (Chawla and Prasad, 1974)in laboratory to estimate initial root-knot nematode population in the field. Enriched FYM was applied at the time of sowing in furrows. Okra variety Gujarat Anand Okra 5 was seeded in furrows. All recommended agronomic practices were followed.

Periodic picking of okra fruits was done and finally fruit yield was recorded. After 3 months of sowing, the experiment was terminated by removing the plants from the field and roots were washed gently under running tap water. Observations on Initial nematode population/200 cm^3 soil,Plant stand, initial and final/m length, plant height at 30, 60 and 90 DAS,Yield, kg/ha,Root-knot index (0-5 scale), Final nematode population (200 cm^3 soil + 3 g roots) were recorded. Roots were cut in to 2-3 cm length and 3 g roots were stained in 0.05 % acid fuchsin in lactophenol. Then stained roots were washed with tap water to remove excess stain and kept overnight in lactophenol, then the roots were examined for nematode population.At the time of termination of experiment final nematode population per 200 cm^3 soil recorded.

Results and Discussion

Initial nematode population was 247 J_2/200 cc soil in field.Plant height was maximum (21.84 cm) and minimum (15.86 cm) in the treatment of soil application of *P. lilacinum* (T_1) and control (T_7) respectively. All the treatments and control were statistically at par with each other (Table 1). The results presented in Table 1 revealed that all bio control agents were found significantly superior as compared to control (T_7) and FYM (T_6) application.

Plant height

At 30 DAS plant height was maximum (21.84 cm) and minimum (15.86 cm) in the treatment of soil application of *P. lilacinum* (T_1) and control (T_7) respectively. All the treatments and control were statistically at par with each other (Table 1).

At 60 DAS, all bio control agents were found significantly superior as compared to control (T_7) and FYM (T_6) application. Maximum plant height was recorded in *P. lilacinum* (T_1) application; however, it was statistically at par with Pf (T_3, Tv (T_4) and Th (T_5) application. Minimum plant height was recorded in control (T_7) followed by FYM (T_6) application.

At 90 DAS, soil application of *P. lilacinum* (T_1) had significantly more plant height as compared to other treatments. Treatment of *P. fluorescens* (T_3) was found second best however, it remained statically at par with each other. Minimum plant height was recorded in control (T_7).

Root-knot index was significantly less in the treatment of *P. lilacinum* (T_1) as compared to rest of the treatments. Treatment of *P. fluorescens* (T^3) is second best treatment and statistically at par with *P. lilacinum* (T_1). As expected, significantly more root-knot index was recorded in the control. However, it was at par with T_6 i.e. FYM (Table 2).

Number of females was significantly less in the treatment of *P. lilacinum* (T_1) as compared to rest of the treatments. Treatment of *P. fluorescens* (T_3) stood second but differed significantly from other treatments except T_4 and T_5. Control had significantly more no. of females and statistically did not differ from T6. i.e. FYM. Almost similar trend was observed in case of number of juveniles/200 cm^3 soil. Total nematode population was significantly lower in *P. lilacinum* (T_1) as compared to rest of the treatments. Treatment *P. fluorescens* (T_3) was next effective and differed significantly from other treatments. Control had significantly more total nematode population and statistically was at par with T_6. *i.e.* FYM. Overall results showed that *P. lilacinum* is found to be most effective in reducing nematode population and root-knot nematode index and thereby increased plant growth and yield and reduced nematode population followed by *P. fluorescens* and *T. harzianum* (Table 2). Results obtained in this study are also conforming the results reported by Veronica and khan (2015) [10], Anon. (2015), Singh *et al*. (2015) [8], Kannan and Veeravel (2012), Rana *et al*. (2014), Singh *et al*. (2014) [7] and Tariq *et al*. (2013) [9].

Yield was significantly higher (11264 kg/ha) in the treatment of *P. lilacinum* (T_1) as compared to rest of the treatments. Treatment of *P. fluorescens* (T_3) is second best treatment and statistically at par with treatment of *P. lilacinum* (T_1). As expected, significantly less (6398 kg/ha) yield was recorded in the control and it remained at par with T6 i.e. FYM (Table 1).Observations on initial and final plant stand (per m length) for all the treatments were found non significant.

Table 1: Effect of different bio-agents on plant height and yield of okra

Treatments	Plant height, cm DAS			Plant stand/m length		Yield (kg/ha)
	30	60	90	Initial	Final	
T_1 (Pl)	21.84^a	69.89^a	86.16^a	4.75^a	4^a	11264^a
T_2 (Pc)	17.74^a	55.35^{bc}	68.43^b	4.75^a	4^a	8780^c
T_3 (Pf)	20.01^a	60.05^{ab}	78.25^{ab}	4.75^a	4^a	10897^{ab}
T_4 (Tv)	18.18^a	57.08^{ab}	70.16^b	4.75^a	4^a	8850^{bc}
T_5 (Th)	18.23^a	57.66^{ab}	71.60^b	4.75^a	4^a	8912^{bc}
T_6 (FYM)	16.07^a	43.18^{cd}	54.93^c	4.75^a	4^a	6791^d
T_7 (CON)	15.86^a	40.73^d	50.32^c	4.75^a	3.75^a	6398^d
S.Em. ±	1.83	4.05	3.96	0.47	0.44	631.35
C.V. %	20.0	14.8	11.5	19.7	22.3	14.3

Figures indicating common letters do not differ significantly from each other at 5% level of significance according to DNMRT

Table 2: Effect of different bio-agents on multiplication of Meloidogyne spp. on okra.

Treatments	RKI (0-5)*	Nematode population		
		No. of female/3 g roots (Log X)	No. of juveniles/200 cm^3 soil (Log X)	Total
T_1 (Pl)	1.92^{d}	49^{d}	2.05^{c} (112)	2.20^{d} (158)
T_2 (Pc)	3.65^{bc}	259^{b}	2.53^{b} (339)	2.78^{b} (603)
T_3 (Pf)	2.68^{d}	163^{c}	2.14^{c} (138)	2.48^{c} (302)
T_4 (Tv)	3.57^{c}	211^{bc}	2.52^{b} (331)	2.73^{b} (537)
T_5 (Th)	3.56^{c}	178^{c}	2.50^{b} (316)	2.70^{b} (501)
T_6 (FYM)	4.51^{ab}	459^{a}	2.91^{a} (813)	3.11^{a} (1288)
T_7 (CON)	5.00^{a}	505^{a}	2.98^{a} (955)	3.17^{a} (1479)
S.Em. ±	0.27	16.89	0.07	0.05
C.V. %	15.2	13.0	5.2	3.6

*0 = Free; 5 = Maximum disease intensity

Figures in parentheses are re-transformed values of Log X

Figures indicating common letters do not differ significantly from each other at 5% level of significance according to DNMRT

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