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Management of citrus nematode, *Tylenchulus semipenetrans* in acid lime, *Citrus aurantoifolia*

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Abstract
The field experiment was conducted for management of citrus nematode infesting acid lime with six treatments including an untreated control. All the treatments were found to be significantly superior over an untreated control in reducing nematode population, egg masses and number of females at intermediate and termination stage of the experiment. However, the treatment of fluopyram 400SC @ 1250 ml / ha was found to be most effective in reducing the nematode population (48.94 %), number of females (37.70 %) and number of egg masses (56.10 %) at an intermediate stage. The next effective treatment was application of bioagent with *Purpureocillium lilacinum* @ 20 kg / ha. At termination stage of experiment. Treatment of bioagent *viz., Purpureocillium lilacinum* @ 20 kg / ha was found to be highly effective. At termination, the chemical treatment was significantly inferior to the organic treatments.

Keywords: Citrus nematode, *Tylenchulus semipenetrans*, Nematode population, Number of females and Number of egg masses

Introduction
Citrus (*Citrus* spp.) belongs to Rutaceae family are native to a vast region stretching from the Himalayan foothills of Northeast India to North-Central China, the Philippines and parts of Southeast Asia, including Burma, Thailand, Indonesia and New Caledonia. Acid lime (*Citrus aurantifolia*) is native to tropical Southeast Asia. They hold significant economic value due to their widespread cultivation and large- scale production. In India, citrus ranks third in fruit production, following mango and banana (Murkute *et al*., 2005) [13]. Area, Production and Productivity under citrus cultivation in India for the year 2024 was 114.6 Thousand ha, 14575.7 MT and 13.1 MT per ha, respectively and in Maharashtra 0.02 thousand ha, 0.50 MT and 29.47 MT per ha, respectively (FAO, 2024). The area under citrus cultivation is expanding steadily, but the yield has not shown a corresponding increase due to various abiotic and biotic stresses, including insect pests, diseases and plant-parasitic nematodes. Among the different phytonematodes, *Tylenchulus semipenetrans* is responsible for causing slow decline disease. In India, *T. semipenetrans* was initially reported by Siddiqi in 1961 from Aligarh (U.P.) later it was documented in almost all citrus-growing states (Reddy and Singh, 1979) [14]. Nematodes that parasitize plants are the main issue with citrus crop cultivation. *Tylenchulus semipenetrans*, *Xiphinema index*, *Meloidogyne* spp., *Radopholus similis* and *Rotylenchus coffee* are the main nematode pests infesting citrus crops (Kumar and Das, 2019) [10]. At the global level, *T. semipenetrans*, is distributed in various parts of US including California, Texas, Arizona and Florida (Bains and Martin, 1974) [1]. Their infestation is also reported in the Mediterranean region which includes the Mediterranean Basin and South African areas (Inserra and Sinha, 2005) [8]. The citrus nematode, *T. semipenetrans* (Cobb, 1913) [4] is one of the most significant plant-parasitic nematodes affecting citrus plants. It has been reported in every citrus-growing region worldwide (Milne, 1977 and Duncan, 2005) [5]. This nematode-induced disease causes a notable reduction in fruit yield, size and quality (Duncan and Cohn, 1990) [6]. The symptoms of slow decline vary based on the level of infestation, the age of the trees and the time of infection. Newly planted citrus orchards usually does not exhibit symptoms until the *T. semipenetrans* population reaches high levels (2000 or more individuals per 100 cc of soil).

The symptoms become more evident in established orchards, especially when trees are stressed by poor growing conditions, drought or root stunting and decay caused by the nematode. Common symptoms of slow decline include reduced leaf and fruit size, thinning of the canopy and the exposure of bare crown limbs, all of which contribute to reduced yield (Duncan, 2005) [5].

Organic additions in the soil are useful not only for nematode management but also for plant development and productivity. The bioagents, *Pseudomonas fluorescence*, *Purpureocilium lilacinum* and Phule *Trichoderma* were also reported effective for managing the root- knot nematode in grapes (Sundara Babu *et al.*1999 and Chormule *et al.*, 2017) [15, 3] acid lime and pomegranate (Walunj, 2013) [16]. The effectiveness of neem cake was also reported in pomegranate (Kadam, 2014) [9]. Organic substrates, on the other hand, promote the growth of beneficial microflora in the rhizosphere, which helps to lower the number of plant parasitic nematodes in the soil. The use of nematode resistant cultivars is another excellent option. They are a cost-effective and environment friendly and manage nematode with a higher yield than infected sensitive crop cultivars.

Material and Methodology

Material

The laboratory appliances and other materials required during the course of investigation *viz.* petri-plates, test tubes, conical flask, beaker, measuring cylinder, pipette, cavity block, Stereoscopic and compound microscope, sieves (20, 60, 200, 350 mesh), lacto phenol, acid fuchsin etc. For different experiments, glassware were cleaned with qualitative liquid soap then rinsed by cold water and finally rinsed with distilled water. At last all the glassware were dipped in potassium dichromate solution ($K_2Cr_2O_7$ 60g, conc. H2SO4 6ml, distilled water 1L) for overnight and rinsed in tap water and dried in hot air oven before use. Glassware were sterilized at 180°C for two hours in hot air oven.

Table 1: Treatment details

Sr. No.	Bionematicide/ Nematicide	Available conc. Cfu/g	Trade name	Biological strain / Chemical name
1.	*Purpureocillium lilacinum*	(2 x 10^6)	Bio-cure	*Purpureocillium lilacinum*
2.	*Pseudomonas fluroscens*	(2 x 10^6)	Phule Sufluero 0.5% WP	*Pseudomonas fluroscens*
3.	*Trichoderma viride*	(2 x 10^6)	Phule Trichoderma 1% WP	*Trichoderma viride*
4.	Neem cake	N:3%, P:1%, K:1%	Neem Strong	Azadirachtin, Salannin, Meliantriol
5.	Fluopyram	400 SC	Velum Prime	N-[2chloro (trifluoromethyl)- 2 pyridyl ethyl]- trifluoroortho-toluamide

Methodology

Soil sample collection

In each treatment, 500 g composite soil-root samples were collected at 30 cm depth from four sides of a tree, each 45-60 cm apart from the tree trunk, at the beginning and at the end of the experiment. Soil from different locations within each site was collected and mixed thoroughly and composite samples was brought to the laboratory to count the nematode population, number of females and egg masses.

Isolation of Nematodes

Nematodes were wet screened from the soil samples by a combination of Cobb's sieving and decanting technique (Cobb, 1913) [4] and Modified Baermann's Funnel Technique. First the soil from the polythene bag was spread on the table, mixed well and most of the debris as well as roots were removed. Then about 200cc of soil sample was taken into Plastic pan, one litre of water was added and stirred well in order to prepare a soil suspension. The remaining clods were broken by hands while removing more of plant debris and gravels. The suspension was allowed to stand for about 10 seconds so that sand and heavier particles settled down. The muddy soil suspension was passed through an assembly of phosphoro-bronze wire-netted sieves of 20, 60, 200 and 350 mesh sieves. Residues of 20 and 60 mesh sieve were washed out. Contents of 200 and 350 mesh sieve was collected into a 200cc beaker by backwashing the sieve with gentle stream of water from the tap. Each soil sample was wet screened in the same manner to collect most of the nematodes. The whole suspension collected in the beaker was poured onto a double layer tissue paper resting on a supporting wire-gauge matching the petri dish. The wire gauge assembly was then placed on petri dish containing sufficient water such that the bottom of the wire gauge tissue paper assembly was slightly submerged in water. The assembly was covered by lid to prevent loss of water due to evaporation and left as such for 24 hours, so as to allow maximum number of nematodes to wriggle out through the tissue paper into the bottom of petri dish. Next day, the wire gauge was removed. 1 ml suspension was taken out with the help of micro pipette in counting dish, put on the stage of the stereoscopic microscope to examine the presence of nematodes. In this manner all samples screened were processed following modified Baermann's Funnel Technique to get clear nematode suspension.

$$\text{Population Density} = \frac{\text{Number of Nematodes}}{\text{Total Number of Samples}}$$

$$\text{Nematode occurrence percentage} = \frac{\text{Number of positive samples}}{\text{Total number of samples}}$$

Result and Discussion

Management of citrus nematode, *Tylenchulus semipenetrans* in acid lime, *Citrus aurantifolia*

The various management approaches have generally been found to be useful in nematode control. In order to assay the effect of different treatments for the management of nematode, *Tylenchulus semipenetrans* infesting citrus cv. Phule Sharbati, a field experiment was conducted on naturally nematode infested orchard at All India Coordinated Research Project on Fruits, Department of

Horticulture, MPKV, Rahuri. The observations on soil population of citrus nematode/200 cc soil, number of females and egg masses/5 g of roots were recorded before the commencement of experiment. Similarly, these observations were recorded at intermediate and termination stage of the experiment. Based on these observations per cent reduction in nematode population, number of females and egg masses were worked out.

Effect of different treatments on citrus nematode population (J2)/200cc of soil

The pre-treatment nematode population in the field plots was within the range of 455 to 485 nematodes (J2) /200 cc of soil. It was observed from the data depicted in Table 1. that all the treatments were significantly superior over an untreated control in reducing the nematode population at intermediate and termination stage of the experiment.

The treatment of Fluopyram 400 SC @ 1250 ml /ha was found to be significantly superior over untreated control in reducing nematode population to 48.94 per cent, at the intermediate stage of the experiment which was at par with Neem cake @ 2 ton/ ha which recorded 45.83 per cent reduction in nematode population, *Purpureocillium lilacinum* @ 20 kg/ ha recording 44.90 per cent decline in nematode population and *Pseudomonas fluorescens* @ 20 kg / ha recording 43.96 per cent decline in nematode population. Least effective nematode control was observed in treatment of *Trichoderma viride* @ 20 kg/ha which recording 38.46 per cent decline in nematode population.

At termination of the experiment, the treatment with bioagent *Purpureocillium lilacinum* @ 20 kg/ha was found highly effective recording 41.84 per cent decline in nematode population. The next effective treatment was neem cake @ 2 ton/ha recording 35.42 per cent decline in nematode population which is at par with *Pseudomonas fluorescens* @ 20 kg/ha and *Trichoderma viride* @ 20 kg/ha recording 35.16 and 31.87 per cent decline in nematode population, respectively. Fluopyram 400 SC @ 1250 ml/ha was found least effective recording 19.15 per cent decline in nematode population.

Table 2: Effect of different treatments on nematode population in citrus

Sr. No.	Treatment	Dose/ha	Nematode population (J) /200cc			Per cent decline in nematode population *	
			Initial	Intermediate	Termination	Intermediate	Termination
1.	Neem cake	2 tons	480	260	310	45.83 (42.62)	35.42 (36.53)
2.	*Pseudomonas fluorescens* 0.5%WP ($2x10^6$ cfu/g)	20 kg	455	255	295	43.96 (41.54)	35.16 (36.38)
3	*Purpureocillium lilacinum* 1%WP ($2x10^6$cfu/g)	20 kg	490	270	285	44.90 (42.08)	41.84 (40.31)
4	*Trichoderma viride* 1%WP (2x 10^6 cfu/g)	20 kg	455	280	310	38.46 (38.34)	31.87 (34.38)
5	Fluopyram 400SC	1250 ml	470	240	380	48.94 (46.22)	19.15 (25.95)
6	Untreated Control	-	485	515	545	0.00 (0.00)	0.00 (0.00)
	S.E. ±	-	12.14	9.17	8.11	1.7	1.11
	CD. at 5%	-	NS	28	25	5.1	3.43
	C.V. ±	-	5.14	7.16	5.23	7.70	5.25

*****Figures in parentheses are arc sin transformed value

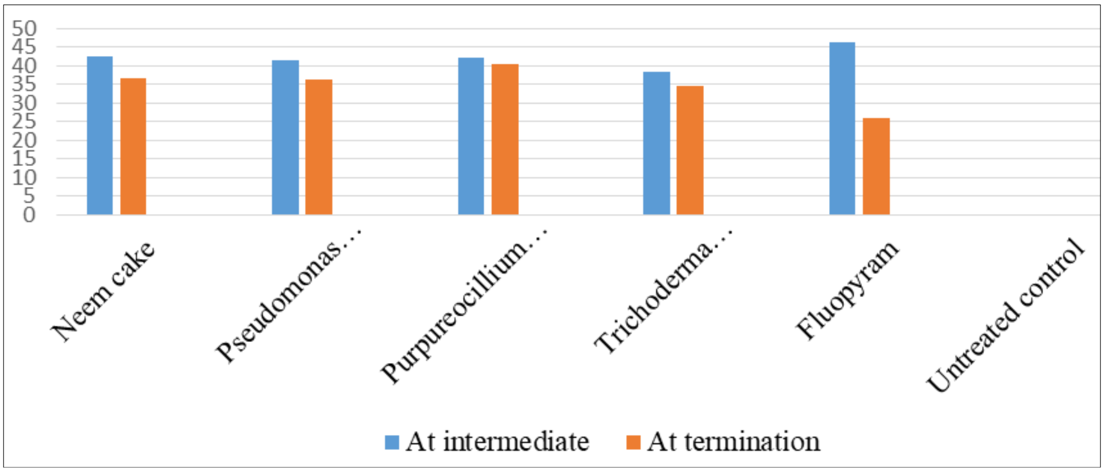

Fig 1: Per cent decline in nematode population over control

Effect of different treatments on number of females/5 g roots

According to the data presented in Table 2 it was observed that all the treatments were significantly superior in reducing the number of females over untreated control at intermediate and at termination stage of the experiment. The treatment of fluopyram 400 SC @ 1250 ml /ha was found to be significantly superior in reducing number of females/ 5 g of roots to 37.70 per cent, at the intermediate stage of the experiment. The next treatment in the order of effectiveness was soil application of bioagents *Pseudomonas fluorescens* @ 20 kg/ha recording 30.25 per cent decline in number of females/5g roots which was at par with *Purpureocillium lilacinum* @ 20 kg/ha, neem cake @ 2t/ha and *Trichoderma viride* @ 20kg/ha recording 29.37, 27.42 and 26.05 per cent decline in number of females/5g root, respectively. At termination of the experiment, the treatment of bioagents *viz*., *Purpureocillium lilacinum* @ 20 kg per ha was found highly effective in recording number of females/5 g roots by recording 26.98 per cent reduction in female population which is at par with *Trichoderma viride* @ 20 kg/ha and neem cake @ 2 t/ha recording 23.53 per cent and 23.39 per cent decline in female population respectively followed by *Pseudomonas fluoroscens* @ 20 kg/ha which recorded 18.49 per cent decline in female population. The least effective treatment observed at termination was fluopyram 400 SC @ 1250 ml/ha which recorded 16.39 per cent reduction in number of females/5 g of roots.

Table 3: Effect of different treatments on number of females /5g roots

Sr. No.	Treatment	Dose/ha	Number of females / 5 g of roots			% Decline in number of females *	
			Initial	Intermediate	Termination	Intermediate	Termination
1.	Neem cake	2 tons	31.00	21.00	23.50	27.42 (31.58)	23.39 (28.92)
2.	*Pseudomonas fluorescens* 0.5%WP (2x10^6 cfu/g)	20 kg	29.75	20.75	22.75	30.25 (33.37)	18.49 (25.47)
3	*Purpureocillium lilacinum* 1%WP (2x10^{6}cfu/g)	20 kg	31.50	21.50	22.25	29.37 (32.81)	26.98 (31.30)
4	*Trichoderma viride* 1%WP (2x 10^6 cfu/g)	20 kg	29.75	22.00	23.50	26.05 (30.69)	23.53 (29.02)
5	Fluopyram 400SC	1250 ml	29.00	19.75	25.50	37.70 (37.88)	16.39 (23.88)
6	Untreated Control		31.25	32.00	34.25	0.00 (0.00)	0.00 (0.00)
	S.E. ±	-	0.80	0.55	0.68	1.44	1.3
	CD. at 5%	-	NS	1.70	2.10	4.44	4.07
	C.V. ±	-	5.32	5.19	5.76	8.68	9.33

* Figures in parantheses are arc sin transformed value

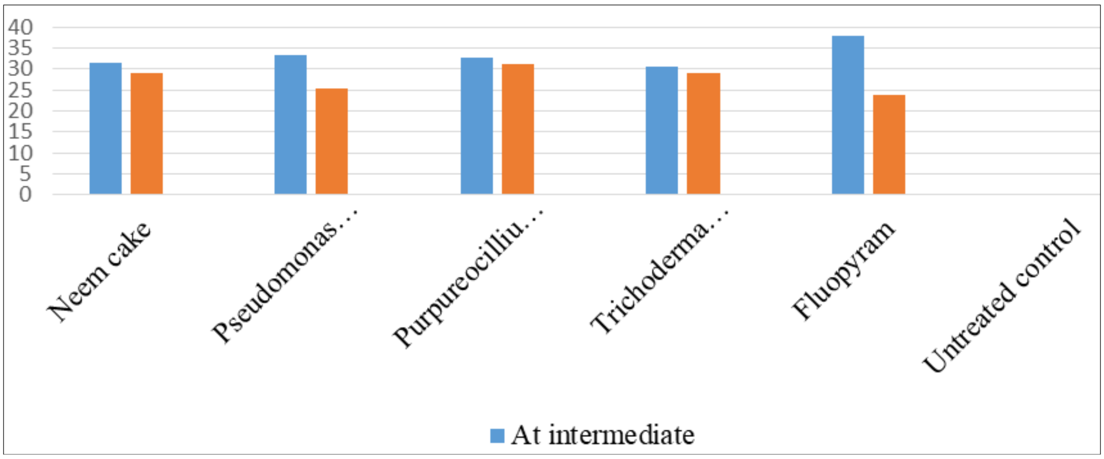

Fig 2: Per cent decline in number of females over control

Effect of different treatments on number of egg masses of nematode / 5 g of roots

It was observed from the data given in Table 4.10 that all the treatments were significantly superior over untreated control in reducing the egg masses produced by the citrus nematode at intermediate and at termination stage of the experiment. The treatment of fluopyram 400 SC @ 1250 ml /ha was found to be significantly superior in reducing number of egg masses/5 g of roots by 56.10 per cent, at the intermediate stage of the experiment which is at par with neem cake @ 2 t/ha recording 47.62 per cent decline in number of egg masses. The next treatment in the order of effectiveness was soil application of *Purpureocillium lilacinum* @ 20 kg/ ha recording 41.86 per cent decline which is at par with *Pseudomonas fluorescens* @ 20 kg/ha and *Trichoderma viride* @ 20 kg/ ha recording 41.77 per cent decline in number of egg masses.

At termination of the experiment, the treatments with bioagents *viz*., *Purpureocillium lilacinum* @ 20 kg / ha, was found highly effective recording 43.02 per cent decline in egg masses per 5 g roots which is at par with neem cake @ 20 kg/ha recorded 35.71 per cent decline in number of egg masses / 5 g of roots. The next effective treatments of *Pseudomonas fluorescens* @ 20 kg/ha recording 35.44 per cent decline in number of egg masses/5 g of roots which was at par with *Trichoderma viride* @ 20 kg/ha recording 31.65 per cent decline in number of egg masses. The least effective treatment observed at termination was fluopyram 400 SC which recorded 24.39 per cent reduction in number of egg masses/5 g of roots.

Table 4: Effect of different treatments on number of egg masses / 5g roots

Sr. No.	Treatment	Dose/ha	Number of egg masses / 5 g of roots			% Decline in number of egg masses *	
			Initial	Intermediate	Termination	Intermediate	Termination
1.	Neem cake	2 tons	21.00	11.00	13.50	47.62 (43.64)	35.71 (36.70)
2.	*Pseudomonas fluorescens* 0.5%WP ($2x10^6$ cfu/g)	20 kg	19.75	10.75	12.75	41.77 (40.27)	35.44 (36.54)
3	*Purpureocillium lilacinum* 1%WP ($2x10^6$cfu/g)	20 kg	21.50	11.50	12.25	41.86 (40.32)	43.02 (40.99)
4	*Trichoderma viride* 1%WP ($2x\ 10^6$ cfu/g)	20 kg	19.75	12.00	13.50	41.77 (40.27)	31.65 (34.23)
5	Fluopyram 400SC	1250 ml	20.05	9.75	15.50	56.10 (48.50)	24.39 (29.60)
6	Untreated Control		21.25	22.00	24.25	0.00 (0.00)	0.00 (0.00)
	S.E. ±	-	0.60	0.54	0.40	1.71	1.39
	CD. at 5%	-	NS	1.68	1.25	5.20	4.30
	C.V. ±	-	6.12	10.0	6.0	8.0	7.61

* Figures in parantheses are arc sin transformed value

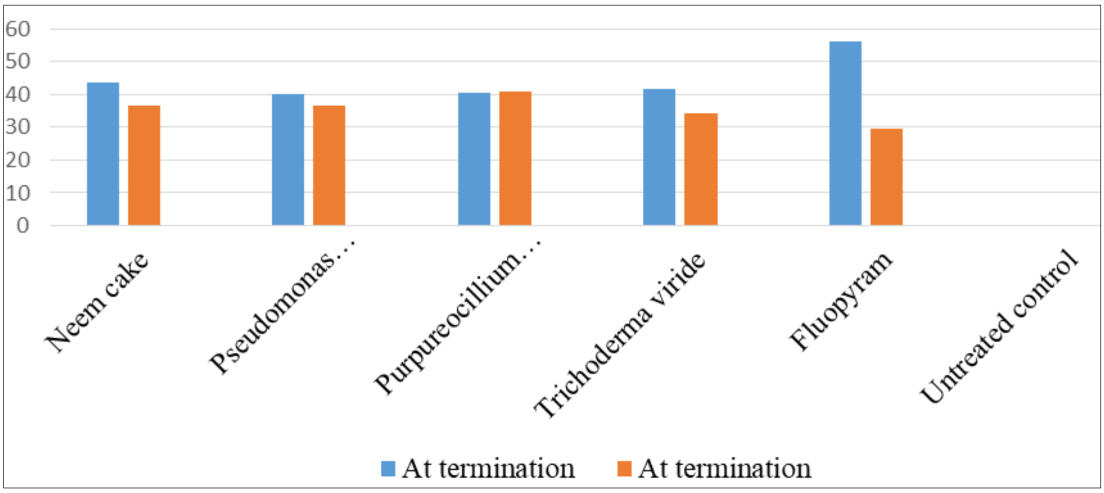

Fig 3: Per cent decline in number of egg masses over control

It could be concluded that at an intermediate stage, fluopyram 400 SC showed highest per cent reduction in nematode population (48.94 %), number of female (37.70 %) and egg masses (56.10 %) and thus proved to be the most effective treatment at intermediate stage. At termination of experiment, treatment of bioagent *viz.*, *Purpureocillium lilacinum* @ 20 kg / ha was found most effective recording 41.84, 26.98 and 43.02 per cent decline in nematode population, number of females and egg masses per 5g roots, respectively. Neem cake showed persistent effect from intermediate to termination of the experiment. This property of neem cake is due to presence and slow release of active ingredients like nimbidine and thionimone which are toxic to nematode. Symptoms due to association of nematode were found negligible. However, treatments employed for nematode management were also found effective against citrus nematode *viz.*, bioagents, organic amendments and nematicide fluopyram was found useful. This findings is in agreement with Baniya *et al.* (2025) [2] who reported that *Tylenchulus semipenetrans* was effectively controlled by using parasitic fungi, *Purpureocillium lilacinum* and similar results were recorded by Maznoor *et al.* (2002) [11] who evaluated the biopotential of *Peacilomyces lilacinus* (now *Purpureocillium lilacinum*) at different inoculum levels. Results revealed that lowest nematode population, number of females and number of egg masses/5 g of roots were found effective in treatment of *Purpureocillium lilacinum* which recorded decline in nematode population (41.84 %), decline in number of females/5 g of roots (26.98 %) and decline in number of egg masses/5 g of roots (43.02 %). These result supports the present research findings.

Effect of different treatments on yield of acid lime and their incremental cost benefit ratio

It is observed from the yield data that all the treatments induced the significant effect on increase in the yield of acid lime. Among the different treatments, soil application of *Purpureocillium lilacinum* @ 20 kg/ha was found to be significantly superior in recording highest yield of acid lime 8.69 ton/ha as against 7.25 ton/ ha in untreated control and recorded 1:9.46 ICBR in this treatment.

Table 5: Effect of different treatments on yield of acid lime and their incremental cost benefit ratio

Tr. No.	Treatment	Gross return (Rs./ha)	Yield (ton/ha)	Additional yield over control (ton/ha)	Additional Profit (Rs.)	Plant protection cost (Rs./ha)	Net profit (Rs./ha)	ICBR
		A	B	C	D	E	F=D-E	G=F/E
T1	Neem cake	8.50	425000	1.25	62500	23000	39500	1:1.72
T2	*Pseudomonas Fluoroscens*	8.07	403500	1.32	41000	6500	34500	1:5.31
T3	*Purpureocillium lilacinum*	8.69	434500	1.94	68000	6500	61500	1:9.46
T4	*Trichoderma viride*	8.14	407000	1.39	44500	6500	38000	1:5.85
T5	Fluopyram 400 SC	8.14	407000	1.39	44500	10000	34500	1:3.45
T6	Untreated control	7.25	362500	-	-	-	-	-

Conclusion
The field experiment conducted for the management of nematode infesting citrus showed that treatment of nematicide Fluopyram 400 SC @ 1250 ml /ha was found to be significantly superior at intermediate stage in reducing nematode population (48.94%). At the termination of experiment, treatments of *Purpureocillium lilacinum* @ 20kg / ha, was found highly effective in reducing nematode population (41.84%).

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