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# Management of Citrus nematode, *Tylenchulus semipenetrans* through chemigation with liquid formulations of *Purpureocillium lilacinum* and neem in acid lime orchards

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**ABSTRACT:** Field experiments were conducted to study the individual and combined effect of neem seed kernel extract (NSKE) and liquid *Purpureocillium lilacinum* formulation delivered through drip irrigation system on acid lime trees naturally infested with citrus nematode, *Tylenchulus semipenetrans***.** Both bio-products were applied at 4 L/ha, but NSKE alone delivered twice at 30 days interval and their effect was compared with carbofuran 3G at 100 g/tree as spot application. Results revealed that both NSKE and *P. lilacinum* tested significantly suppressed *T. semipentrans* density in soil and acid lime roots. However, *T. semipenetrans* suppression was significantly superior in acid lime trees when NSKE and *P. lilacinum* were concomitantly delivered. This combined treatment reduced *T. semipenetrans* density in soil by 78.0-88.8% and in roots by 73.5-79.2%. The density of T. *semipenetrans* was comparatively higher when inputs were applied alone. Root colonization and egg parasitization potential of *P. lilacinum* was improved 2-3 times more when delivered along with NSKE. The integrated approach caused 26.3-33.6% more fruit yield with higher (1:3.2-1:4.2) cost benefit ratio. Hence, chemigation with NSKE + *P. lilacinum* in acid lime trees can be recommended for wide scale adoption to manage citrus nematodes.

**Keywords:** Citrus, *Tylenchulus semipenetrans,* NSKE, *Purpureocillium lilacinum,* nematode, biopesticide

## INTRODUCTION

Citrus group is the third important fruit crop after mango and banana in India. India produces 4.79 million tonnes of orange fruits annually from 0.62 million ha cultivation area (Anonymous, 2018). Tamil Nadu is one of the leading producers of citrus which grow in 10,297 ha and produce 24,766 MT. The citrus nematode, *Tylenchulus semipenetrans* is the key nematode pest on citrus and responsible for 'slow decline' disease. In India, it is present in more than 90% of the citrus orchards and citrus nurseries (Reddy, 1996). The citrus nematodes primarily infect feeder roots that affect uptake of water and soil nutrients. The nematode infected plants yield less number of fruits with small size. The estimated yield loss due to citrus nematode is 30-50%. The nematode affected citrus trees are more prone to fungal diseases such as *Fusarium solani, Fusarium oxysporum* and *Phytophthora parasitica* which lead to a destructive nematode-disease complex (Begum et al. 2012).

Soil fumigation with methyl bromide or metham sodium is the widely studied methods for the management of *T. semipenetrans* in citrus worldwide. Non-fumigant nematicides such as oxamyl, aldicarb, ethoprophos, fenamiphos, carbofuran and cadusafos have also been tried to contain *T. semipenetrans* populations (Verdejo-Lucas and McKenry, 2004). However, citrus nematode management strategies moved towards complete or partial exclusion of chemicals due to high cost, persistence, phytotoxicity and toxicity to non-target organisms including human toxicity. In addition, frequent applications are required to maintain their populations below threshold levels. In these situations, eco-friendly alternative strategy likes application of organic products and biocontrol agentsis gaining much importance.

Till now various biocontrol agents and bio-products such as *Glomus mosseae* (O'bannon *et al*., 1979); *Pseudomonas fluorescens, Trichoderma viride, Trichoderma harzianum and Pochonia chlamidosporia* (Deepa *et al*., 2011),*Paecilomyces lilacinus* (Parvatha *et al*., 1991),*Pasteuria* spp (Sorribas *et al*., 2000), chitin (Spiegel *et al*., 1989), neem, karanj and castor oil cakes (Reddy *et al*., 1996), glucosinolates purified from brassicaceous plant tissues (Zasada and Ferris, 2003),and leaf extracts of neem, Calotropis and Datura alpha (Ahmad *et al*., 2004) have been evaluated against *T.*

*semipenetrans* as either soil application or seedling bare root dip treatment. However, the scenario of adoption of drip irrigation system by the citrus growers necessitates new approaches for nematode management. Using irrigation systems to apply fertilizers and pesticides is commonly referred to as chemigation. Drip irrigation system provides an efficient vehicle for controlled delivery of bio-products in the soil root zone and success has been achieved on several crops for nematode control (Selvaraj *et al*., 2014; Nagachandrabose, 2020). Since the citrus nematodes complete their life cycle in rhizosphere, the prospects for release of liquid organic products through drip irrigation system which is delivered at the rhizosphere, are very promising. Additionally, less labour is required to apply the bio-formulations.

Hence preliminarily, various liquid formulations of organic products and biocontrol agents at different dosages in acid lime orchards were evaluated. The results revealed that delivery of neem seed kernel extract (NSKE) at 4 L/ha twice at 30 days interval and liquid *P. lilacinum* formulation at 4 L/ha were the better treatments that have reduced *T. semipenetrans* populations significantly (Unpublished). However, *T. semipenetrans* population reduction achieved from these treatments was less than 35%. Also adoption of a single bio-product or biocontrol agent is not ideal for management of *T. semipenetran s*as the left out population after bio-product or biocontrol application invade the fresh roots rapidly and multiply very fast due to high reproduction potential (O'Bannon and Essar, 1994). Hence studies were conducted to investigate the effect of integrating the above components for the sustainable management of *T. semipenetrans* in citrus.

## MATERIALS AND METHODS

### Experiment details

A field experiment was conducted in a farmers acid lime orchard (cv. Kanapathy Local) at Viralimalai village, Pudukottai District, Tamil Nadu during 2019-2020. The acid lime trees were grown at 3 x 3 m spacing and the age of the trees weresixwhich were naturally infested with *T. semipenetrans*. The initial population of nematode was 329 -361 nematodes/ 200 $cm^3$ soil and 50 – 65 females/g of root.The treatments consisted of $T_1$ – Neem seed kernel extract 5% @ 4 L/ha twice at 30 days interval; $T_2$ – Liquid *Purpureocillium lilacinum* @ 4 L/ha; $T_3$ – $T_1$ + $T_2$; $T_4$ – Carbofuran @ 100 g/plant (Standard check); and $T_5$ – Control. The trial was laid in randomised block design and all treatments were replicated four times. Each replicated rows consisted of seven trees.

Concurrently an another identical field experiment with similar treatment schedule was performed at Singarakottai village, Vedachandur Block, Dindigul district, Tamil Nadu, India.The treatments were imposed on 9 year old acid lime trees cv. PKM1 naturally infested with *T. semipenetrans*. The initial population of nematode was 239-248 nematodes/ 200 $cm^3$soil and 35.2-37.6females/g of root. The tree spacing was 5 x 5 m. At location II, each replicated rows consisted of nine trees.

In both fields, NSKE and *P. lilacinum* treatments were delivered through drip irrigation system. A 12 mm drip lateral line was placed for each row of trees. A 2 L/h capacity dripper was provided for each plant. Drippers were fixed at 30 cm away from tree trunk. NSKE and *P.lilacinum* treatments were applied through the venturi. Three rows of acid lime trees under each treatment were given simultaneously. The valves of other tree rows were closed during delivery of particular treatment.

### Experimental inputs

Neem seed kernel extract (NSKE) was prepared as per TNAU standard procedure (Anonymous, 2020). The seed coat removed shade dried neem kernal were used. Five kg of the kernel was pound gently and tied loosely in a cotton cloth. The cloth with kernel was soaked in 10 l water and incubated for 24 h. After incubation, the kernel suspension was filtered. The 500 ml of the kernel suspension was mixed with 9.5 L of water to prepare 5% neem seed kernel extract and used for the field experiments. The liquid formulation of *P. lilacinum* ($5 \times 10^9$ CFU/mL) was obtained from Horticultural College and Research Institute, Periyakulam, Tamil Nadu. NSKE and *P. lilacinum* were mixed with 500 L water and applied through drip irrigation system. The chemical standard check treatment Carbofuran 3G @ 100 g/plant was applied as spot application manually. All other normal horticultural practices were followed in the acid lime trial orchard as per TNAU - Crop Production Guide – 2020 (Anonymous, 2020).

### Nematode density assessment

The nematode population in soil was assessed before treatment and during harvest. Samples of five trees from each replicate were pooled and a sub-sample of 200 $cm^3$was used for nematode extraction. The acid lime tree roots collected along with soil samples were separated and a sub-sample of 1 g root was used for assessment of *T. semipenetrans* female populations. Cobb's decanting and sieving technique followed by modified Baermann's funnel technique was followed for extraction of second stage juveniles (J2) of *T. semipenetrans*from soil samples (Southey, 1986). The female population from a gram root

**Table1. Effect of liquid P. lilacinum and NSKE on T. semipenetrans infestation and infection on acid lime cv. Kanapathy at Location I**

| Treatment | J2 population /200 $cm^3$ soil | | Female populations /g root | |
|---|---|---|---|---|
| | Before treatment | At harvest | Before treatment | At harvest |
| NSKE | 347 | 438b | 50 | 112 b |
| P. lilacinum | 329 | 391b | 65 | 104b |
| NSKE + P. lilacinum | 338 | 104d | 56 | 28d |
| Carbofuran | 341 | 234 c | 54 | 59 c |
| Control | 361 | 932a | 61 | 223a |

Means followed by the same letter in columns are not significantly different at P<0.05 according to Duncans Multiple range test.

**Table 2. Effect of liquid *P. lilacinum* and NSKE on yield of acid lime cv.Kanapathy infected with T. *semipenetrans* at Location I**

| Treatments | No. of fruits/ tree | Fruit weight (g) | Yield (kg / tree) | Yield (ton/ha) | B:C ratio |
|---|---|---|---|---|---|
| NSKE | 204c | 44.4 b | 9.8 c | 8.33 c | 2.6 |
| P. *lilacinum* | 214c | 45.3b | 9.9 c | 8.41 c | 2.7 |
| NSKE + P. *lilacinum* | 294a | 52.6a | 13.1 a | 11.13 a | 3.2 |
| Carbofuran | 236 b | 47.3 b | 11.2 b | 9.52 b | 2.4 |
| Control | 142d | 40.7 c | 8.7 d | 7.39 d | 1.9 |

Means followed by the same letter in columns are not significantly different at P<0.05 according to Duncans Multiple range test.

**Table 3. Effect of liquid P. *lilacinum* and NSKE on T. *semipenetrans* infestation and infection on acid lime cv. PKM1 at Location II**

| Treatments | J2 population /200 $cm^3$ soil | | Female population /g root | |
|---|---|---|---|---|
| | Before treatment | At harvest | Before treatment | At harvest |
| NSKE | 245 | 82 d | 35.6 | 16.4 d |
| P. *lilacinum* | 242 | 142 c | 37.6 | 22.0 c |
| NSKE + P. *lilacinum* | 248 | 62 e | 36.4 | 9.2 e |
| Carbofuran | 246 | 182 b | 35.2 | 26.4 b |
| Control | 239 | 282 a | 36.4 | 45.6 a |

Means followed by the same letter in columns are not significantly different at P<0.05 according to Duncans Multiple range test.

was counted under stereozoom microscope after stained with acid fuchsin-lactophenol.

**Re-isolation of *P. lilacinum***

Root samples from the five labelled trees were collected during harvest and cut in to 1 cm length bits. One gram of sub-samples was used for isolation of the *P. lilacinum*. Root bits were dipped in 1% NaOCl for surface sterilization and rinsed twice in distilled water. The rinsed root bits were taken in a pestle and mortar and ground by adding 1 mL of distilled water. Then 9 mL of sterile distilled water was added in the ground suspension. Similarly, serial dilutions of the suspension up to $10^{-7}$were prepared and 100 µL aliquot of the $10^{-3}$to$10^{-7}$dilutions were placed on potato dextrose agar. Five Petri dishes were used for each dilution and incubated at 29±3°C for 14 days and fungal colonies were counted in terms of CFU.

**Egg mass parasitization**

Percentage egg mass parasitization was assessed for *P. lilacinum*. Ten egg masses from root sub-samples of each tree were hand-picked and rinsed with sterile distilled water twice and plated on potato dextrose agar media in 90-mm Petri plates. The plates were incubated at 29 ±3°C for 14 days and fungi emerging from each egg mass were identified. The percentage of *P. lilacinum* parasitization was calculated using the formula: (number of egg masses infected with *P. lilacinum*/total number of egg mass) × 100.

**Yield parameters**

Yield parameters such as number of fruits/tree, fruit weight, fruit yield/tree and yield/ha were recorded.

**Data analysis**

Cost effectiveness of each treatment was assessed based on Cost-benefit (CB) ratio.CB ratio was the ratio of total benefit divided by total cost. All the data collected were analyzed using analysis of variance and means separated with the Duncan Multiple Range Test following Panse and Sukhatme (1989). The software used was AGRES.

**RESULTS**

The population density of *T. semipenetrans* J2 in acid lime trees before treatment was not significantly different in experiment trees in both location I and II (Tables 1 and 3). The *T. semipenetrans* infestation range was 329 -361 J2 per 200 $cm^3$ soil in Location I and 239-248 J2 per 200 $cm^3$ soil in Location II. However, *T. semipenetrans* density during harvest was significantly different among treatments in both locations ($P < 0.001$). The density ranged from 104 to 938 J2 per 200 $cm^3$ soil in Location I and 62 to 282 J2 per200 $cm^3$ soil in Location II. Integration of NSKE and *P. lilacinum*had significantly lesser population of *T. semipenetrans* in location I and II ($P < 0.05$). Combined treatment reduced the *T. semipenetrans* density by 88.8% in Location I and 78.0% in Location II. The next best nematode control effect was noted in carbofuran at Location I that caused 74.8% reduction of *T. semipenetrans* density and in NSKE at Location II with 70.9% reduction. Combined treatment was performed consistently better than carbofuran treatment in both locations.

All the bio-inputs and carbofuran (chemical check) significantly reduced the *T. semipenetrans* infection on acid lime roots ($P < 0.001$). However, there was a significant variation among the inputs to suppress the *T. semipenetrans* infection ($P < 0.05$). The higher suppression was noticed when integrating NSKE + *P. lilacinum* delivery, which reduced *T. semipenetrans* by 73.5% in Location I and 79.2% in Location II. The root population of *T. semipenetrans* was comparatively higher when inputs individually applied.

In *P. lilacinum* introduced trees, it colonized acid lime roots and remained up to harvest (Table 5). However, colonization potential of *P. lilacinum* significantly improved when delivered along with NSKE. The colony count of *P. lilacinum* was 2.8 - 3.0 x $10^8$ CFU when combined with NSKE whereas 0.9 – 1.0 x $10^8$ CFU during solo delivery. The egg parasitization by *P. lilacinum* was also 2.6-3.1 times more when applied along with NSKE.

Yield of acid lime trees in terms of number of fruits and fruit weight significantly increased by all bio-input treatments. The number of fruits in untreated trees was significantly less in both locations (Tables 2 and 4). Combination of NSKE + *P. lilacinum* treatment had significantly higher number of fruits than all other treatments. The fruits in NSKE + *P. lilacinum* delivered trees were 34.7-51.7% more with 22.6-34.4% more weight. The yield improvement was lesser in carbofuran and individual bio-input treated trees. The improvement in yield characters of NSKE + *P. lilacinum* trees resulted in significant increase in acid lime yield. The maximum yield increase was noticed in the trees treated with NSKE + *P. lilacinum* (26.3-33.6%) followed by carbofuran (13.9-14.4%). The results on economics revealed that NSKE + *P. lilacinum* treatment recorded higher (1:3.2-1:4.2) cost benefit ratio.

**Table 4. Effect of liquid *P. lilacinum* and NSKE on yield of acid lime cv.PKM1 infested with *T. semipenetrans* at Location II.**

| Treatments | No. of Fruits/tree | Fruit Weight (g) | Yield (kg/ tree) | Yield (ton/ha) | B:C ratio |
|---|---|---|---|---|---|
| NSKE | 832 b | 41.2 b | 30.2 b | 12.08 b | 3.7 |
| Purpureocillium | 800 c | 38.6 c | 29.6 b | 11.84 cb | 3.5 |
| NSKE + Purpureocillium | 986 a | 45.9 a | 37.2 a | 14.88 a | 4.2 |
| Carbofuran | 735 d | 35.4 d | 32.0 c | 12.80 b | 3.2 |
| Control | 643 e | 30.1 e | 27.4 d | 10.96 d | 2.3 |

Means followed by the same letter in columns are not significantly different at P<0.05 according to Duncans Multiple range test.

**Table 5. Root colonization and egg parasitization of introduced P. *lilacinum* on acid lime roots infected with T. *semipenetrans***

| Treatment | Root colonization of introduced *P. lilacinum* (CFU x $10^5$ $g^{-1}$ root) | | Egg mass parasitisation (%) | |
|---|---|---|---|---|
| | Location I | Location II | Location I | Location II |
| NSKE | - | - | - | - |
| *Purpureocillium* | 1086 b | 982 b | 19.7 b | 22.6 b |
| NSKE + *Purpureocillium* | 3021 a | 2815 a | 61.3 a | 58.1 a |
| Carbofuran | - | | - | - |
| Control | - | | - | - |

Means followed by the same letter in columns are not significantly different at P<0.05 according to Duncans Multiple range test.

## DISCUSSION

It is evident that delivery of NSKE and *P. lilacinum* through drip system in acid lime resulted significant reduction of *T. semipenetrans* population. The nematode control potential of NSKE was established against *Meloidogyne incognita*, *Meloidogyne javanica*, *Radopholus similis*, *Pratylenchus goodeyi* and *Rotylenchulus reniformis* (Javed *et al*., 2008; Seenivasan *et al*., 2013; Gupta *et al*., 2020). Effect of NSKE was not yet studied on *T. semipenetrans*, though other neem products such as leaf extract and oil cake were evaluated against them (Ahmad *et al*., 2004; Bamel and Sonkar, 2013).The reduction of *T. semipenetrans* infestation and infection in citrus trees by NSKE might be due to its three established mode of actions such as i) direct toxicity or nematicidal action to juveniles and adults stages of nematode, ii) nematostatic effect on nematode, iii) egg hatch inhibition (Kapil *et al*., 1994; Kosma *et al*., 2011; Ladi *et al*., 2019). The azadirachtin and other chemical compounds such as alkaloids, saponins, triterpenoids, steroids, tanins, phenol, anthocyanins, flavonoids, glycosides present in NSKE also proved to possess nematicide, nematostatic and egg inhibition action against nematodes (Seenivasan, 2010; Kosma *et al*., 2011).

Mani *et al*. (1989) established the lethal effect of *P. lilacinum* on second stage juveniles of *T. semipenetrans* under *in vitro* and proved its parasitization effect on adult females and egg mass of *T. semipenetrans* in citrus seedlings. Field efficacy of *P. lilacinus* multiplied or formulated on oil cake, rice bran and talc powder was established earlier (Maznoor *et al*., 2002; Seenivasan, 2011; Basati and Kaul, 2015). This study demonstrated the liquid formulation is also effective against nematodes under field condition. The results of the present study are in confirmatory with Nagachandrabose (2018) who reported that the pathogenicity of *P. lilacinum* against *M. hapla* was better as liquid formulations. Conidia and sclerotia of *P. lilacinum* are more stable, viable for long period and resistant to desiccation stress (Song *et al*., 2016).

The population reduction was prominent in acid lime trees when both NSKE and *P. lilacinum* were applied together. Moreover, the root colonization and egg parasitization potential of *P. lilacinum* was increased when delivered along with NSKE. Our result is in agreement with Parvatha *et al*. (1991) who recorded maximum reduction of *T. semipenetrans* due to integrated application of *P. lilacinus* and neem cake. Combination of *P. lilacinum* with neem leaf aqueous suspension led to a complementary effect such as more colonization of egg plant roots and higher *M. incognita* egg parasitization (Rao and Reddy, 2001). Nagesh *et al*. (2003) observed the improved propagules of *P. lilacinum* in chrysanthemum rhizosphere with 52% more egg mass parasitization due to combined use with neem cake. Although many organic amendments and oil cakes were tried along with *P. lilacium*, its efficacy was remarkably increased when integrated with neem products (Ahmad and Khan, 2004).

Drastic reduction of *T. semipenetrans* infestation and infection on citrus trees in NSKE + *P. lilacinum* treatment reflected on significantly increased lime fruit yield. More number of heavier fruits in both field trials might also been due to growth promoting effect or fertilizer value of NSKE and *P. lilacinum*. NSKE is rich in plant nutrients such as nitrogen (5.5-7.1), phosphorus (1.1%) and potash (1.5%) (Ketkar, 1976). The *P. lilacinum* has the capacity to make soil phosphorus into plant available form (Lima-Rivera *et al*., 2016). NSKE also has the capacity to release nitrogen slowly by inhibition of soil nitrification process through its triterpenes metabolite (Akhtar, 1998).

It is concluded that sequential delivery of neem seed kernel extract 5% @ 4 L/ha twice at 30 days interval + Liquid *Purpureocillium lilacinum* @ 4 L/ha was the most promising technology as revealed by*T. semipenetrans* infestation / infection suppression, fruit yield improvement and profitable returns in acid lime. This integrated management technology can be popularised among citrus growers for the management of citrus nematodes.

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## REFERENCES

Ahmad, M.S., Mukhtar, T. and Ahmad, R. 2004. Some Studies on the control of citrus nematode (*Tylenchulussemipenetrans*) by leaf. *Asian Journal of Plant Science*, **3**(5): 544-548.

Akhtar, M.1998. Biological control of plant-parasitic nematodes by neem products in agricultural soil. *Applied Soil Ecology*, **7**(3):219-223.

Anonymous, 2018. Horticulture Statistics at a Glance – 2018.Controller of Publications, Government of India. p. 458. (Available online:http://agricoop.nic.in/sites/default/files/Horticulture%20Statistics%20at%20a%20Glance-2018.pdf )

Anonymous, 2020. Crop production guide – Horticulture crops. Available online: http://autoshelltnau.in/research/wp-content/uploads/sites/60/2020/02/Horticulture-CPG-2020.pdf

Bamel, V. and Sonkar, R.K. 2013. Effect of phorate 10G and neem cake on *Tylenchulus semipenetrans* in a declining Nagpur Mandarin orchard in Central India. *Indian Journal of Nematology*, **43**(1):70-73.

Basati, J.K. andKaul, V.K. 2015. Efficacy of *Paecilomyces lilacinus* for the management of citrus nematode, *Tylenchulus semipenetrans*. *Plant Disease Research*, **30**(2): 156-158.

Begum, N., Haque, M.I., Mukhtar, T., Naqvi, S.M. and Wang, J.F. 2012. Status of bacterial wilt caused by *Ralstonia solanacearum* in Pakistan. *Pakistan Journal of Phytopathology*, **24**(1):11-20.

Deepa, S.P., Subramanian, S. and Ramakrishnan, S. 2011. Biomanagement of citrus nematode *Tylenchulus semipenetrans* Cobb on lemon, *Citrus limonia* L. *Journal of Biopesticides*, **4**(2): 205.

Gupta, H., Kumar, S. and Sharma, R. (2020) Eco-friendly management of root-knot nematode, *Meloidogyne incognita* (Kofoid& White) chitwood using seed kernel extracts, cow urine and agniastra. *Journal of Entomology and Zoological Studies*, **8**(2):1115-1118.

Javed, N., Gowen, S.R., El-Hassan, S.A., Inam-ul-Haq, M., Shahina, F. and Pembroke, B. 2008. Efficacy of neem (*Azadirachtaindica*) formulations on biology of root-knot nematodes (*Meloidogyne javanica*) on tomato. *Crop Protection*, **27**:36-43.

Kapil, A., Sharma, S. and Wahidulla, S. 1994. Leishmanicidal activity of 2 Benzoxazolinone from *Acanthus illicifoliusin vitro*. *Planta Medicine,* **60**:187.

Ketkar, C.M. 1976. Utilization of neem *(Azadirachta indica* Juss.) and its bye-products. Report of the modified neem cake manurial project: 1969-1976. Khadi Village Industries Commission, Bombay, India.p. 234.

Kosma, P., Ambang, Z., Begoude, B.A.D., Ten Hoopen, G.M., Kuaté, J. and Akoa, A. 2011. Assessment of nematicidal properties and phytochemical screening of neem seed formulations using *Radopholus similis* parasitic nematode of plantain in Cameroon. *Crop Protection*, **30**(6):733-738.

Lima-Rivera, D.L., Lopez-Lima, D., Desgarennes, D., Velazquez-Rodriguez, A.S. and Carrion, G. 2016. Phosphate solubilization by fungi with nematicidal potential. *Journal of Soil Science and Plant Nutrition*, **16**(2): 507-524.

Maznoor, S., Sinha, A.K. and Bora, B.C. 2002. Management of citrus nematode, *Tylenchulus semipenetrans* on Khasi Mandarin, by *Paecilomyces lilacinus*. *Indian Journal of Nematology*, **32**(2): 153-155.

Musabyimana, T. and Saxena, R. 1999. Efficacy of neem seed derivatives against nematodes affecting banana. *Phytoparasitica* **27**(1): 43-49.

Nagachandrabose, S. 2018. Liquid bioformulations for the management of root-knot nematode, *Meloidogyne hapla* that infects carrot. *Crop Protection*, **114**: 155-161.

Nagachandrabose, S. 2020. Management of potato cyst nematodes using liquid bioformulations of *Pseudomonas fluorescens, Purpureocillium lilacinum* and *Trichoderma viride*. *Potato Research*, 1-18.

Nagesh, M., Hussaini, S.S., Singh, S.P. and Biswas, S.R. 2003. Management of root-knot nematode, *Meloidogyne incognita* (Kofoid& White) Chitwood in chrysanthemum using *Paecilomyces lilacinus* (Thom) Samson in combination with neem cake. *Journal of Biological Control*, **17**(2): 125-132.

O'Bannon, J.H., and Essar, R.P. 1994. *Citrus Declines Caused by Nematodes in Florida I. Soil Factors*. University of Florida Cooperative Extension Service, Institute of Food and Agriculture Sciences, EDIS.

O'bannon, J.H., Inserra, R.N., Nemec, S., and Vovlas, N. 1979. The influence of Glomus mosseae on Tylenchulus semipenetrans-infected and uninfected *Citrus limon* seedlings. *Journal of Nematology*, **11**(3), 247.

Panse, V.G. and Sukhatme, P.V. 1989. Statistical methods for agricultural workers. Indian Council of Agricultural Research, New Delhi, 359.

Parvatha, R., Khan, R.M., and Rao, M.S. 1991. Integrated management of the citrus nematode Tylenchulus semipenetrans using oil-cakes and Paecilomyces lilacinus. *Afro-Asian Journal of Nematology*, **1**(2): 221-222.

Rao, M.S. and Reddy, P. 2001. Control of *Meloidogyne incognita* on egg plant using *Glomus mosseae* integrated with *Paecilomyces lilacinus* and neem cake. *Nematologia Mediterranea,* **29**:153-157

Reddy, P.P., Rao, M.S. and Nagesh, M. 1996.Management of citrus nematode, *Tylenchulus semipenetrans*, by integration of *Trichoderma harzianum* with oil cakes. *Nematologia Mediterranea*, **24**(2): 265-267.

Seenivasan, N. 2010. Management of root-knot nematode, *Meloidogyne incognita* with organic amendments in medicinal Coleus. *Annals of Plant Protection Sciences*, **18**(2): 472-476.

Seenivasan, N. 2011. Efficacy of *Pseudomonas fluorescens* and *Paecilomyces lilacinus* against *Meloidogyne graminicola* infecting rice under system of rice intensification. *Archives of Phytopathology and Plant Protection*, **44**(15): 1467-1482.

Seenivasan, N., Manoranjitham, S.K., Auxilia, J. and Soorianathasundaram, K. 2013. Management of nematodes in banana through bio-rationale approaches. *Pest Management in Horticultural Ecosystems*, **19**(1): 38-44.

Selvaraj, S., Ganeshamoorthi, P., Anand, T., Raguchander, T., Seenivasan, N. and Samiyappan, R. 2014. Evaluation of a liquid formulation of *Pseudomonas fluorescens* against *Fusarium oxysporumf. sp. cubense* and *Helicotylenchus multicinctus* in banana plantation. *Bio Control*, **59(**3): 345-355.

Song, Z., Shen, L., Zhong, Q., Yin, Y. and Wang, Z. 2016. Liquid culture production of microsclerotia of *Purpureocillium lilacinum* for use as bionematicide. *Nematology*, **18**(6): 719-726.

Sorribas, F.J., Verdejo-Lucas, S., Forner, J.B., Alcaidel, A., Pons, J. and Ornat, C. 2000. Seasonality of *Tylenchulus semipenetrans* Cobb and *Pasteuria* sp. in citrus orchards in Spain. *Journal of Nematology*, **32**(4S): 622.

Southey, J.F. 1986. *Laboratory methods for work with plant and soil nematodes*.HMSO.

Spiegel, Y., Cohn, E. and Chet, I. 1989. Use of chitin for controlling *Heterodera avenae* and *Tylenchulus semipenetrans*. *Journal of Nematology*, **21**(3):419

Verdejo-Lucas, S. and McKenry, M.V. 2004. Management of the citrus nematode, *Tylenchulus semipenetrans*. *Journal of Nematology*, **36**(4):424.

Zasada, I.A. and Ferris, H. 2003. Sensitivity of *Meloidogyne javanica* and *Tylenchulus semipenetrans* to isothiocyanates in laboratory assays. *Phytopathology*, **93**(6):747-750.

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