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Bio-efficacy of nano-emulsion formulation of *Purpureocillium lilacinum* against root-knot nematode, *Meloidogyne incognita* on carrot

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Abstract
Pot experiments were conducted to evaluate the bio-efficacy of Nano-emulsion formulation of nematode egg parasitic fungi (*Purpureocillium lilacinum*) at different doses *viz*., 0.25 ml/L, 0.5 ml/L, 1.0 ml/L, 2.5 ml/L and 5.0ml/L against root-knot nematode (*Meloidogyne incognita*) in carrot *var*. TAQ II 99. The results revealed that *P. lilacinum* at doses of 1.0 to 5 ml/L showed superior effect on suppressing the *M. incognita* infestation on carrot in terms of Juvenile (J2) population in soil, female population in roots and root gall index. These treatments reduced the J2 population by 46.42% in soil and female population by 30.85% in roots than untreated control. The *P. lilacinum* at 0.5 ml/L also significantly suppressed *M. incognita* in soil by 40.59% and in roots by 25.47%. The *P. lilacinum* treated carrot plants were with improved growth characters such as highest shoot length, shoot weight, root length and root weight. The study thus showed that the nano-emulsion formulation of *Purpureocillium lilacinum* was effective in the management of *M. incognita* and was also revealed to show better crop health benefits. It is concluded that nano emulsion formulation of *Purpureocillium lilacinum* at the dose of 0.5 ml/L to 5.0 ml/L can be recommended for managing root-knot nematode infestation in carrot crop after validation under field conditions.

Keywords: *Purpureocillium lilacinum*, root-knot nematode, nano-emulsion, carrot

1. Introduction
Carrot (*Daucus carota* L.) belonging to the Apiaceae family is one of the top-ten economically important vegetable crop in the globe (Simon *et al*. 2008) [26]. Carrots are a common cool-season root vegetable grown in temperate nations mostly in the spring and summer, and in tropical regions in the winter. Despite the fact that the greens are edible, the taproot is the most commonly consumed component of a carrot. It is rich source for dietary fibre, vitamins, minerals, carotenoids and antioxidants. Worldwide, it is produced at 24 Mt in an 1.1 million ha area that has global trade value of 100 million US dollars (Simon *et al*. 2008) [26]. Next to China, India is one of the important grower of carrots that grow in 22, 538 ha with an annual production of 4.14 lakh tons in states like Assam, Andhra Pradesh, Haryana, Karnataka, Punjab, Uttar Pradesh and Tamil Nadu. (Devrajan *et al*. 2003; Thamburaj and Singh, 2005) [7, 31].

Root-knot nematodes are severe constraint for carrot production in temperate as well as tropic regions. The northern root-knot nematode, *Meloidogyne hapla* is widely prevalent in temperate carrots whereas *Meloidogyne incognit*a is very common in tropical carrots. *Meloidogyne incognita* can cause seedling death, stunting of young plants, branching of tap roots and galling in older plants, which reduces quality and yield. It damage tap roots by galling, digitation, and constriction, resulting in lower market value (Seenivasan, 2017) [22]. Even low soil densities of *M. incognita* have been reported to reduce marketable root yields (Wesly *et al*. 2021) [32]. On mature carrot, infection by *M. incognita* initially causes small galls to develop on secondary roots. If galling occurs during the seedling stages, the carrot roots can become severely stunted and forked and therefore unmarketable. Severe infections and damage to carrot by *M. incognita* have occurred more frequently in recent years in tropical carrot fields. Hence, root-knot nematode control is important to increase the value of carrot production.

Chemical nematicides and crop rotation are the primary means of management of root-knot nematodes on carrot. Chemicals, mainly carbofuran or phorate, are commonly used to escape from the nematode infestation, but their repeated application leads to nematicide application as uneconomical in addition to environment hazard issues.

Alternative methods of control are therefore needed. Only a few resistant germplasm lines have been identified in carrot, and none of the cultivars currently grown in India have been found to be resistant to *M. incognita* (Gugino *et al*., 2006) [9]. Rotation to a non-host or antagonistic crop is also an effective management strategy against *M. incognita* (Abawi and Widmer, 2000) [1]. However, current crop rotations commonly practiced by vegetable growers, especially in tropical regions, are generally not effective, since many of the crops grown in vegetable rotations (such as tomato, brinjal, bhendi, cabbage, and cauliflower) are susceptible. Hence, there is a need for the development of an eco-friendly nematode management strategy to manage RKN in carrot.

Presently, application of biological control agents is emerging as a promising alternative strategy in the management of nematodes. Among the various bio-control agents reported to manage plant parasitic nematodes, egg parasitic fungus and *Purpureocillium lilacinum* was frequently reported as effective against RKN in various crops including carrot (Nagachandrabose, 2018) [17]. Earlier, talc-based formulations of the above bio-agents have been tried. However, the inferior shelf life trend of talc-based formulations prompted a development of liquid formulations. Advantages of the liquid formulations of bio-agents include high cell count, zero contamination, longer shelf-life, greater protection against environmental stresses, increased field efficacy and convenience in handling (Nagachandrabose, 2020) [18]. Hence, a new nano-emulsion formulation of *Purpureocillium lilacinum* was evaluated in this study to manage *M. incognita* on carrot.

2. Materials and Methods

2.1. *Meloidogyne incognita* culture

The *Meloidogyne incognita* culture used in this study was isolated from carrot cv. TAQ II 99 at Krishnagiri district. Single egg mass from the carrot plant was used to establish pure culture on tomato cv. CO1. The juveniles required for this study was taken from the tomato pure cultures.

2.2. *Purpureocillium lilacinum*

The nano-emulsion formulation of nematode egg parasitic fungi *P. lilacinum* strain TNAUPL1 containing spore load of 1.02×10^5 spores/ml was obtained from the Department of Nano-science and Technology, Tamil Nadu Agricultural University, Coimbatore, India.

2.3. Pot study

Two pot culture trials were conducted in the Department of Nematology, Tamil Nadu Agricultural University, Tamil Nadu, India. Both trials were laid at similar time from April 2022 – July 2022. The carrot cv. TAQ II 99 seeds were used for both pot trials.

Both experiment consisted of the following five treatments: (1) Soil drench (SD) with nano-emulsion of *P. lilacinum* at 0.25 mL/L; (2) SD with nano-emulsion of *P. lilacinum* at 0.50 mL/L; (3) SD with nano-emulsion of *P. lilacinum* at 1.0 mL/L; (4) SD with nano-emulsion of *P. lilacinum* at 2.5 mL/L; (5) SD with nano-emulsion of *P. lilacinum* at 5.0 mL/L and (6) Untreated control. The experiments were laid out in completely randomized block design with five replicates. Five kg soil capacity clay pots containing autoclaved pot mixture soil (sand: red earth: farmyard manure – 2:1:1) considered as one replicate. Nano-emulsion of *P. lilacinum* treatments were drenched before sowing. The three seeds of carrot were sown in each pot. Then *M. incognita* J2 @ 5,000/pot was used as inoculums. The plants were maintained at glass house and irrigated once in a day. Plants were fertilized with 20-20-20 (N-P-K) fertilizer at 0.1 % concentration at twenty days intervals. No pest or disease was recorded during the study.

The plants were carefully uprooted at 60 days after inoculation. The observations on shoot length, shoot weight, root length and root weight were recorded. The root gall index in the scale of 0-5 scale was recorded: 0 = no galls and no forking; 1 = 1-10 galls on secondary roots, taproot not affected; 2 = 11-50 galls, none coalesced, taproot with light forking; 3 = 51-100 galls with some coalesced, forking; 4 = more than 100 galls with many coalesced, severe forking; 5 = more than 100 galls, mostly coalesced, severe forking (Belair and Parent 1996). To estimate J2 population from soil, Soil sample of 200 cm^3 was taken from each pot and was processed by Cobb's sieving technique followed by the modified Baermann funnel method (Southey, 1986) [29]. The population of second stage juveniles (J2) of *M. incognita* was counted under a stereoscope microscope at 40x magnification. Then secondary roots from each plant were collected, cut into 1 cm length, stained with acid fuschin-lactophenol and the adult females were counted under a stereo zoom microscope (Seenivasan and Devrajan, 2008) [21].

2.4. Statistical analysis

In the present study, the data produced from several experiments were statistically analyzed. For statistical analysis, AGRES software was used to calculate the mean values.

3. Results

Results of Experiment 1 showed that soil drenching with nano-emulsion formulation of *P. lilacinum* significantly reduced *M. incognita* and improved the growth of carrot. There was significant difference exist among the test doses evaluated. The J2 population in soil ranges from 98 to 243. The population reduction was directly proportionate to test doses. Test doses of 1 ml/L, 2.5 ml/L and 5 ml/L recorded significantly least population (97-101 J2/200 cm^3) followed by 0.5 ml/L and 0.25 ml/L. The female population per gram of root ranged from 7 to 70. The female populations significantly high in untreated plants. The *P. lilacinum* drenching resulted on 52.8 to 75.7% reduction of female population on roots. Female population reduction was significantly high in 1 ml/L, 2.5 ml/L and 5 ml/L doses. The root gall index was also significantly least (2.0) in these test doses whereas gall index was 3.0 in 0.25ml/L and 0.5 ml/L doses. The gall index was 5.0 in untreated plants. The *P. lilacinum* parasitized the *M. incognita* eggs 63-85% when applied at 0.25 ml/L to 5 ml/L. The parasitization was significantly high at 1 ml/L, 2.5 ml/L and 5 ml/L doses. The *P. lilacinum* also parasitized the adult females of *M. incognita*. Female parasitization was least (55.8%) at 0.25 ml/L dose and high (62.3%) at 5 ml/L (Table. 1).

The reduction on J2 population in soil, female population and root gall index lead to improved growth of carrot plants. The shoot length was ranged from 19.78 to 47.78 cm. The pots drenched with *P. lilacinum* at 1 ml/L, 2.5 ml/L and 5 ml/L recorded significantly more shoot length (45.54-47.78 cm). The shoot length also significantly high in 0.25 ml/L and 0.5 ml/L doses than untreated plants. The shoot length was significantly least (19.78 cm) in untreated plants. The shoot weight of the different treatment also significantly differed

with least shoot weight (9.87 g) in untreated plants. The shoot weight of *P. lilacinum* treatment found to improve from 34.0 to 64.7%. Shoot weight was significantly high in *P. lilacinum* treatments at ml/L, 2.5 ml/L and 5 ml/L. The lower dose treatments 0.25 ml/L and 0.5 ml/L also recorded significantly high shoot weight than untreated control. The plants received *P. lilacinum* had significantly more root length than untreated control. The *P. lilacinum* doses 1 ml/L, 2.5 ml/L and 5 ml/L recorded significantly high root length followed by 0.5 and 0.25 ml/L doses. Similar trend was observed for root weight. The root weight ranged from 20.77 to 34.08 g among treatments. *P. lilacinum* treatments improved the root weight from 15.4 to 39.0% with high improvement in 1 ml/L, 2.5 ml/L and 5 ml/L doses (Tables. 2).

Similar trends were seen in the results of Experiment II, where soil drenching with *P. lilacinum* nano-emulsion formulation greatly reduced *M. incognita* and enhanced carrot growth. The test doses examined varied significantly from one another. It was found that the J2 population in the soil ranges from 100 to 248. It was found that the population reduction was directly proportional to test doses. Following 0.5 ml/L and 0.25 ml/L in terms of much lower population were test dosages of 1 ml/L, 2.5 ml/L, and 5 ml/L (100-102 J2/200 cm3). There were 18 to 72 females per gram of root. In untreated plants, the female population was significantly high. The female population on roots was reduced by 53.7 to 76.8 percent as a result of the nano-emulsion formulation *P. lilacinum* drenching. The female population reduction in the 1 ml/L, 2.5 ml/L, and 5 ml/L dosages was noticeably significant. In comparison to the test doses of 0.25 ml/L and 0.5 ml/L, the root gall index was much lower (2.0) in these doses. The gall index for untreated plants was 5.0. When used at 0.25 ml/L to 5 ml/L, *P. lilacinum* parasitized the *M. incognita* eggs in 67–92% of cases. At concentrations of 1 ml/L, 2.5 ml/L, and 5 ml/L, the parasitization was significantly high. The adult females of *M. incognita* were likewise parasitized by *P. lilacinum*. At doses of 0.25 ml/L and 5 ml/L, female parasitization was lowest (56.70 percent) and highest (63.78 percent), respectively (Table. 3).

Carrot plants grew more quickly as a result of lower J2 populations in the soil, female populations, and root gall indices. 20.12 to 46.32 cm was the range for the shoot length. *P. lilacinum* at concentrations of 1 ml/L, 2.5 ml/L, and 5 ml/L significantly increased the shoot length in the pots (45.15-46.32 cm). In comparison to untreated plants, the shoot length was also significantly higher at 0.25 and 0.5 ml/L doses. In untreated plants, the shoot length was notably the lowest (20.12 cm). The four treatments recorded shoot weights also significantly varied, with untreated plants having the lowest shoot weight (10.77 g).The *P. lilacinum* treatment was observed to increase shoot weight from 33.5 to 63.2 percent. In *P. lilacinum* treatments at ml/L, 2.5 ml/L, and 5 ml/L, shoot weight was notably high. Compared to the untreated control, the lower dose treatments (0.25 ml/L and 0.5 ml/L) also produced considerably higher shoot weights. In comparison to the untreated control, the plants that received *P. lilacinum* exhibited considerably longer roots. Following the 0.5 and 0.25 ml/L dosages, the *P. lilacinum* doses of 1 ml/L, 2.5 ml/L, and 5 ml/L reported considerably high root length. The trend for root weight was similar. Between treatments, the root weight varied between 19.86 and 33.96 g. Treatments with *P. lilacinum* increased the root weight from 15.0 to 38.6 percent, with significant improvements seen in doses of 1 ml/L, 2.5 ml/L, and 5 ml/L (Table. 4).

Table 1: Effect of nano-formulation of *P. lilacinum* on *M. incognita* infection on carrot cv. TAQ II 99 (Experiment I).

Treatments	Juveniles (J2) /200g of soil	Females/ g of root	Root gall index	Egg parasitization (%)	Adult Female parasitization (%)
T1 – Nano-formulation of *Purpureocillium lilacinum* @ 0.25ml/L	152.00^{c}	33.00^{c}	3d	63.00^{c}	55.85
T2 - Nano-formulation of *Purpureocillium lilacinum* @ 0.5ml/L	115.00^{b}	29.00^{b}	3^{d}	74.00	57.14
T3 - Nano-formulation of *Purpureocillium lilacinum* @ 1.0ml/L	101.00^{a}	18.00^{a}	2^{d}	87.00^{a}	60.00
T4 - Nano-formulation of *Purpureocillium lilacinum* @ 2.5ml/L	97.00^{a}	17.00^{a}	2^{d}	84.00^{a}	62.23
T5 - Nano-formulation of *Purpureocillium lilacinum* @ 5.0ml/L	98.00^{a}	17.00^{a}	2^{d}	85.00	62.30
T6 - Untreated Control	243.00^{d}	70.00^{d}	5^{d}	0.00^{d}	0.00^{f}

Values are mean of 4 replications. In a column, means followed by common alphabet are significantly different from each other at 5% level by Duncan's Multiple Range Test (DMRT).

Table 2: Effect of nano-formulation of *P. lilacinum* on growth parameters of carrot infected with *M. incognita* (Experiment I).

Treatments	Shoot length (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)
T1 – Nano-formulation of *Purpureocillium lilacinum* @ 0.25ml/L	33.74^{c}	18.46^{c}	9.85^{c}	24.57^{c}
T2 - Nano-formulation of *Purpureocillium lilacinum* @ 0.5ml/L	38.75^{b}	21.94^{b}	11.23^{b}	28.44^{b}
T3 - Nano-formulation of *Purpureocillium lilacinum* @ 1.0ml/L	45.54^{a}	25.85^{a}	13.96^{a}	32.33^{a}
T4 - Nano-formulation of *Purpureocillium lilacinum* @ 2.5ml/L	47.67^{a}	27.93^{a}	15.25^{a}	34.01^{a}
T4 - Nano-formulation of *Purpureocillium lilacinum* @ 5.0ml/L	47.78^{a}	28.01^{a}	15.32^{a}	34.08^{a}
T6 - Untreated Control	19.78^{d}	9.87^{d}	7.02^{d}	20.77^{c}

Values are mean of 4 replications. In a column, means followed by common alphabet are significantly different from each other at 5% level by Duncan's Multiple Range Test (DMRT).

Table 3: Effect of nano-formulation of *P. lilacinum* on *M. incognita* infection on carrot cv. TAQ II 99 (Experiment II).

Treatments	Juveniles (J2) / 200g of soil	Juveniles (J2) / g of root	Root gall index	Egg parasitization (%)	Adult Female parasitization (%)
T1 – Nano-formulation of *Purpureocillium lilacinum* @ 0.25ml/L	157.00^{c}	25.00^{c}	3d	67.00^{c}	56.70
T2 - Nano-formulation of *Purpureocillium lilacinum* @ 0.5ml/L	118.00^{b}	22.00^{b}	3^{d}	78.00	58.25
T3 - Nano-formulation of *Purpureocillium lilacinum* @ 1.0ml/L	102.00^{a}	19.00^{a}	2^{d}	90.00^{a}	61.12
T4 - Nano-formulation of *Purpureocillium lilacinum* @ 2.5ml/L	100.00^{a}	18.00^{a}	2^{d}	92.00^{a}	63.34
T5 - Nano-formulation of *Purpureocillium lilacinum* @ 5.0ml/L	100.00^{a}	18.00^{a}	2^{d}	92.00^{a}	63.78
T6 - Untreated Control	248.00^{d}	72.00^{d}	5^{d}	0.00^{d}	0.00^{f}

Values are mean of 4 replications. In a column, means followed by common alphabet are significantly different from each other at 5% level by Duncan's Multiple Range Test (DMRT).

Table 4: Effect of nano-formulation of *P. lilacinum* on growth parameters of carrot infected with *M. incognita* (Experiment II).

Treatments	Shoot length (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)
T1 – Nano-formulation of *Purpureocillium lilacinum* @ 0.25ml/L	34.58^{c}	18.12^{c}	10.02^{c}	23.27^{c}
T2 - Nano-formulation of *Purpureocillium lilacinum* @ 0.5ml/L	39.13^{b}	21.03^{b}	12.08^{b}	27.48^{a}
T3 - Nano-formulation of *Purpureocillium lilacinum* @ 1.0ml/L	45.15^{a}	24.66^{a}	14.35^{a}	31.70^{a}
T4 - Nano-formulation of *Purpureocillium lilacinum* @ 2.5ml/L	46.27^{a}	26.81^{a}	16.44^{a}	33.92^{a}
T5 - Nano-formulation of *Purpureocillium lilacinum* @ 5.0ml/L	46.32^{a}	26.88^{a}	16.47^{a}	33.96^{a}
T6 - Untreated Control	20.12^{d}	10.77^{d}	6.18^{d}	19.86^{c}

Values are mean of 4 replications. In a column, means followed by common alphabet are significantly different from each other at 5% level by Duncan's Multiple Range Test (DMRT).

4. Discussion

The development of bio-management techniques using egg parasitic fungus is an emerging field in crop protection to reduce the economic loss caused by plant parasitic nematodes In the current study, *P. lilacinum* showed greater antagonistic activity against *M. incognita* under glass house conditions. The results are consistent with the findings of several research workers who demonstrated the use of *P. lilacinum* against various plant parasitic nematodes (Singh *et al*. 2013; Hore *et al*. 2018; Dahlin *et al*. 2019) [27, 11, 5]. The *P. lilacinum* strain used in this study had already been reported to protect potato plants from *Globodera rostochiensis* (Nagachandrabose, 2020) [18], slow decline of citrus caused by *Tylenchulus semipenetrans* (Seenivasan *et al*. 2020) [24], root-knot disease of tomato caused by *Meloidogyne incognita* (Das and Waquar, 2021) [6], root lesion disease of sugarcane by *Pratylenchus zeae* (Jayakumarand Seenivasan, 2020) and tuberose root-knot disease (Rajamanickam and Ravindran, 2021) and northern root-knot nematode, *M. hapla* (Kalaiarasan *et al*. 2017) [14]. Numerous modes of action have been postulated and demonstrated for antagonistic effects of *P. lilacinum* in controlling plant parasitic nematodes. Primarily, *P. lilacinum* antagonize *M. incognita* by parasitizing or by direct hyphal penetration of eggs, egg mass and also the developing adult females. The metabolites like acetic acid, eucinostatins and paecilotoxin were reported to produce by *P. lilacinum* that can help to kill or prevent the free living populations in soil before root penetration (Singh *et al*. 2013) [27]. The roots colonized by *P. lilacinum* was also reported to arrest the formation of nurse cells or giant cells by nematodes (Cabanilla *et al*. 1988) [4].

Results of this study also showed that soil drenching with *P. lilacinum* improved the growth of carrot plants in terms of shoot length, shoot weight, root length and root weight. Similar improvement of plant growth characters like shoot height, root length and weight was established in pineapple plants after *P. lilacinum* application (Kiriga *et al*. 2018) [15]. The growth promotion by *P. lilacinum* through phosphorus solubilization as well as IAA production was reported by (Baron *et al*., 2020) [2]. Similar mechanisms might be the reason for the improved growth of carrot plants after *P. lilacinum* drenching in our study.

Nano-emulsion formulation of *Purpureocillium lilacinum* were used as bio-control agents in our study and their beneficial effects on plant growth was studied. Nano-emulsions were developed to enhance the bioavailability of bioactive substances ingested with them (McClements *et al*., 2016) [16]. *P. lilacinum* has the capacity to establish well in the soil over a short period of time and become the dominant species where it is treated (Siddiqui & Mahmood, 1996) [25]. In our study, the nano-emulsion formulation of our fungal bio-agent had produced best results in terms of plant growth parameters and nematode reproduction. The fact that nano-emulsion formulation could produce the desired result might have been due to the enhanced dosage at the field level and also the method of applying bio-agents also would have been crucial in nematode suppression in host plants. *P. lilacinum* conidia and microsclerotia were found to be more viable, resilient to stresses such desiccation, and stable in the liquid condition, which may be the causes of the increased pathogenicity (Song, Shen, Zhong, Yin, & Wang, 2016) [28]. So it could have resulted in lowering the nematode population. Various studies have been conducted to commercialize nano-formulation in the agricultural sector but it still depends on how well the preparation and application of nano-emulsion gets carried out in the lab so that it can be scaled up while going for commercial usage. A few more studies also supported *P. lilacinum* field effectiveness when used as a soil treatment to prevent *M. incognita* in tomato plants (Singh, Pandey, & Goswami, 2013) [27]. The current study provides evidence that *P. lilacinum* nano-formulation efficiently lowers the *M. incognita* population, which in turn improves carrot growth and yield.

Results of this study clearly evidenced that nano-emulsion formulation of *P. lilacinum* is effective against *M. incognita* on carrot. This is a first report of positive effect of nano-formulation of *P. lilacinum*. Earlier, *P. lilacinum* was formulated on solid substrate such as millet grains or oil cakes like neem derivatives (Jonathan and Rajendran, 2000) [13]. Later, it was formulated on talc powder carriers and tested on different crop plants (Singh *et al*. 2013) [27]. In recent years, liquid formulation of *P. lilacinum* was found effective against various nematodes on diverse crops (Seenivasan, 2018; Nagachandrabose, *et al*. 2022) [19]. In the earlier studies, the dosage required to get desired nematode control was at 5 ml/L concentration. However, the nano-emulsion formulation reported in this study was required at low concentrations to get desired nematode control. The concentration of 0.5 ml/L is sufficient to get more than 60% egg parasitization. Hence, it is concluded that soil drenching with 0.5 ml/L to 5 ml/L of *P. lilacinum* can be recommended for the control of root-knot nematodes infesting the carrot.

5. References

1. Abawi GS, Widmer TL. Impact of soil health management practices on soilborne pathogens, nematodes and root diseases of vegetable crops. Applied soil ecology. 2000;15(1):37-47.
2. Baron NC, de Souza Pollo A, Rigobelo EC. *Purpureocillium lilacinum* and *Metarhizium marquandii* as plant growth-promoting fungi. PeerJ. 2020;8:e9005.
3. Belair G, Parent LE. Using crop rotation to control *Meloidogyne hapla* Chitwood and improve marketable carrot yield. HortScience. 1996;31(1):106-108.
4. Cabanillas E, Barker KR, Daykin M. Histology of the interactions of *Paecilomyces lilacinus* with *Meloidogyne incognita* on tomato. Journal of Nematology. 1988;20(3):362.
5. Dahlin P, Eder R, Consoli E, Krauss J, Kiewnick S. Integrated control of *Meloidogyne incognita* in tomatoes using fluopyram and *Purpureocillium lilacinum* strain 251. Crop Protection. 2019;124:104874.
6. Das N, Waquar T. Bio-Efficacy of *Purpureocillium lilacinum* on Management of Root-Knot Nematode, *Meloidogyne incognita* in Tomato. Indian Journal of Nematology. 2021;51(2):129-136.
7. Devrajan K, Seenivasan N, Selvaraj N. Bio-management of root-knot nematode, *Meloidogyne hapla*, in carrot (*Daucus carota* L.). Indian Journal of Nematology. 2003;33(1):6-8.
8. Ekanayake HMRK, Jayasundara NJ. Effect of *Paecilomyces lilacinus* and *Beauveria bassiana* in controlling *Meloidogyne incognita* on tomato in Sri Lanka. Nematologia Mediterranea, 1994, 87-88.
9. Gugino BK, Abawi GS, Ludwig JW. Damage and management of *Meloidogyne hapla* using oxamyl on carrot in New York. Journal of Nematology. 2006;38(4):483.
10. Hano P, Khan MR. Evaluation of fungal (*Paecilomyces lilacinus*) formulations against root knot nematode infecting tomato. Bangladesh J Bot. 2016;45(5):1003-1013.
11. Hore J, Roy K, Maiti AK. Evaluation of Bio-Nematon (*Purpureocillium lilacinum* 1.15% WP) against root-knot nematode (*Meloidogyne incognita*) in tomato. J Entomol Zool Stud. 2018;6(4):1700-1704.
12. Jayakumar J, Seenivasan N. Eco-friendly management of Sugarcane nematode in Field. Annals of Plant Protection Sciences. 2020;28(3):251-255.
13. Jonathan EI, Rajendran G. Bio-control potential of the parasitic fungus *Paecilomyces lilacinus* against the root-knot nematode *Meloidogyne incognita* in banana. Journal of Biological Control. 2000;14(2):67-69.
14. Kalaiarasan P, Swarnakumari N, Poornima K. Management of northern root knot nematode, *Meloidogyne hapla* in carrot (*Daucus carota* L.) with fungal bio-agents along with organic amendment. Pest Management in Horticultural Ecosystems. 2017;23(1):101-103.
15. Kiriga AW, Haukeland S, Kariuki GM, Coyne DL, Beek NV. Effect of *Trichoderma* spp. and *Purpureocillium lilacinum* on *Meloidogyne javanica* in commercial pineapple production in Kenya. Biological Control. 2018;119:27-32.
16. McClements DJ, Saliva-Trujillo L, Zhang R, Zhang Z, Zou L, Yao M, *et al*. Boosting the bioavailability of hydrophobic nutrients, vitamins, and nutraceuticals in natural products using excipient emulsions. FoodResearch International. 2016;88:140-152.
17. Nagachandrabose S. Liquid bio-formulations for the management of root-knot nematode, *Meloidogyne hapla* that infects carrot. Crop Protection. 2018;114:155-161.
18. Nagachandrabose S. Management of potato cyst nematodes using liquid bio-formulations of *Pseudomonas fluorescens*, *Purpureocillium lilacinum* and *Trichoderma viride*. Potato Research. 2020;63(4):479-496.
19. Nagachandrabose S, Jayaraman J, Somasundaram P. Application of liquid bio-inoculants through a drip irrigation system to manage slow decline disease caused by *Tylenchulus semipenetrans* in acid lime trees. Phytoparasitica. 2022;50(1):243-253.
20. Rajamanickam C, Ravindran C. Bio-management of root knot nematode (*Meloidogyne incognita*) in tuberose (*Polianthes* tuberose L.). Journal of Pharmacognosy and Phytochemistry. 2021;10(1):1693-1695.
21. Seenivasan N, Devrajan K. Management of *Meloidogyne incognita* on medicinal coleus by commercial bio-control formulations. Nematologia Mediterranea, 2008.
22. Seenivasan N. Status of root-knot nematode, *Meloidogyne hapla* infection on carrot at Kodaikanal hills of Tamil Nadu, India and its yield loss estimation. Int J Curr Microbiol App Sci. 2017;6(9):3629-3635.
23. Seenivasan N. Effect of concomitant application of *Pseudomonas fluorescens* and *Purpureocillium lilacinum* in carrot fields infested with *Meloidogyne hapla*. Archives of Phytopathology and Plant Protection. 2018;51(1-2):30-40.
24. Seenivasan N, Jayakumar J, Prabhu S. Management of citrus nematode, *Tylenchulus Semipenetrans* through chemigation with liquid formulations of *Purpureocillium lilacinum* and neem in acid lime orchards. Pest Management in Horticultural Ecosystems. 2020;26(2):254-261.
25. Siddiqui ZA, Mahmood I. Biological control of plant parasitic nematodes by fungi: a review. Bio-resource Technology. 1996;58(3):229-239.
26. Simon PW, Freeman RE, Vieira JV, Boiteux LS, Briard M, Nothnagel T, *et al*. Carrot. In Vegetables II (pp. 327-357). Springer, New York, NY, 2008.
27. Singh S, Pandey RK, Goswami BK. Bio-control activity of *Purpureocillium lilacinum* strains in managing root-

knot disease of tomato caused by *Meloidogyne incognita*. Biocontrol Science and Technology. 2013;23(12):1469-1489.
28. Song Z, Shen L, Zhong Q, Yin Y, Wang Z. Liquid culture production of microsclerotia of *Purpureocillium lilacinum* for use as bionematicide. Nematology. 2016;18(6):719-726.
29. Southey JF. Laboratory methods for work with plant and soil nematodes, 1986.
30. Sowmya DS, Rao MS, Kumar RM, Gavaskar J, Priti K. Bio-management of *Meloidogyne incognita* and *Erwinia carotovora* in carrot (*Daucus carota* L.) using *Pseudomonas putida* and *Paecilomyces lilacinus*. Nematologia Mediterranea, 2012.
31. Thamburaj S, Singh N. Textbook of vegetables, tuber crops and spices. New Delhi: Indian Council of Agriculture Research, 2005.
32. Wesly JL, Kalaiarasan P, Devrajan K, Shanthi A, Rajesh S, Elayarajan M. Influence of Edaphic Factors on Root-Knot Nematodes, *Meloidogyne* sp. infesting Carrot (*Daucus carota*) Grown in Tamil Nadu, India. Indian Journal of Nematology. 2021;51(2):166-174.