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Research Article

Exploring the bio-efficacy of biocontrol agents in mitigating *Meloidogyne incognita* menace in carrot cultivation

J. LALSON WESLY[1*], P. KALAIARASAN[2], N. SWARNAKUMARI[2], K. DEVRAJAN[2] and S. G. SHANDEEP[2]
[1]*Division of Nematology, Indian Agricultural Research Institute, Pusa – 110012, New Delhi, India*
[2]*Department of Nematology, Tamil Nadu Agricultural University, Coimbatore – 641003, Tamil Nadu, India*
[*]*Corresponding author Email:* weslyjohnzen@gmail.com

ABSTRACT: The nematicidal efficacy of liquid formulation of *Pochonia chlamydosporia, Bacillus subtilis, Purpureocillium lilacinum, Trichoderma viride* and vermiculite formulation of *Rhizophagus intraradices* were challenged against *Meloidogyne incognita* under glasshouse conditions. The *in vivo* experiment were piloted to test the potential of these biological agents by soil drenching of liquid formulation @ 1 ml/pot or soil application of vermiculite formulation @ 1g/pot/dose. Their effect was compared with the granular application of Carbofuran @ 1g/pot/dose. All the liquid bioformulations investigated were capable of enhancing plant growth and lowering the pathogenicity and parasitic success of *M. incognita* in carrots. The soil drenching of *P. chlamydosporia* caused a significant reduction of galls in the root (51%), nematode population in the root (43.6%), egg mass in the root (65.3%) and infective juvenile population in soil (51.8%) over other treatments and Carbofuran.

KEYWORDS: *Bacillus subtilis,* carbofuran, liquid formulation, *Pochonia chlamydosporia,* root-knot nematode

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INTRODUCTION

The carrot, *Daucus carota* (Apiaceae), stands as a crucial root vegetable globally, displaying a diverse range of colours, shapes and sizes. Initially cultivated for its medicinal properties over 2000 years ago, the diploid *D. carota* (2n=2x=18) has evolved into a dominant and versatile vegetable crop, renowned for its abundant potassium and carotenoids, particularly β carotene, which serves as an antioxidant and a precursor for vitamin A (Potter *et al.,* 2011). On the global scale, India holds the fourth position in terms of carrot cultivation area (119000 Hectares) and the thirteenth position in production (2134000 MT). This achievement is largely attributed to significant contributions from Haryana, Punjab, and Uttar Pradesh (Indiastat, 2018). Specifically, Tamil Nadu, located at the southeast extremity of India, characterized by predominantly tropical regions with a few hills displaying temperate conditions, secures the 8th and 6th ranks nationally in carrot cultivation area and production, respectively (Indiastat, 2022).

In recent times, *Meloidogyne incognita* has emerged as a significant threat to carrot cultivation in the tropical regions of Tamil Nadu. This nematode infestation leads to the deformation and gall formation in carrot roots, rendering them unsuitable for the market (Wesly *et al.,* 2021). Consequently, effective management of the root-knot nematode is imperative to ensure the production of flawless carrots. Several methods can be employed for the management of Plant-Parasitic Nematodes (PPNs), including *M. incognita*. These methods encompass legislation, host plant resistance, cultural practices, physical measures, biological controls and chemical interventions. Each approach has its advantages and disadvantages. Despite the widespread preference among farmers for chemical nematicides due to their quick efficacy, many of these chemicals have been prohibited due to their adverse impacts on human health, beneficial organisms and the environment. (Seenivasan, 2017).

Indeed, Carbofuran @ 1 kg a.i/ha is widely practised by Indian farmers to control PPNs. Carbofuran causes a greater reduction of infective stage juveniles (J_2s) in soil, female infestation in the tuber and number of eggs/g root with enhanced shoot growth and tuber yield (Seenivasan, 2017). However, Carbofuran application influences negatively on fish (Trotter *et al.,* 1991), birds, pets and human beings (Gupta, 1994) causing ecological knockdowns. Thus, Carbofuran 50% SP was banned for import, manufacture and use in India from 2020 (Directorate of Plant Protection Quarantine and Storage, n.d.).

Biological control through verified selection and subsequent introduction of specialized natural enemies particularly microbes against nematodes was considered as one of the important components of sustainable crop production despite its self-propagating and slow reclamation nature (Schroth & Hancock, 1981). The nematode antagonistic microbes are of several types *viz.,* egg parasitizing fungi (*Pochonia chlamydosporia* and *Purpureocilium lilacinum*), nematode-trapping fungi (*Arthobotrys oligospora*, etc.,), endophytic fungi (AM fungi), antibiotic fungi (*Trichoderma* spp.), antibiotic bacteria (*Bacillus* spp.), endospore producing bacteria (*Pasteuria penetrans*) (Perry & Moens, 2006). However, all antagonistic microbes are not mass multiplied due to certain drawbacks, some are commercialized. While some research has explored the nematicidal efficacy of individual bioagents against root-knot nematode in carrots (Sowmya *et al.,* 2012, Bontempo *et al.,* 2014, Prasad *et al.,* 2014, Rao *et al.,* 2017, Viljoen *et al.,* 2019), the comparison between the efficacies of different commercially available nematode antagonistic bioagents are lacking.

Therefore, in the present study, we explored the potential of locally available commercial bio-candidates as alternatives to carbofuran under controlled glasshouse conditions. The aim was to offer an immediate solution to farmers experiencing yield losses attributed to root-knot nematodes in carrot cultivation.

MATERIALS AND METHODS

Species confirmation and pure culture maintenance of *Meloidogyne incognita*

The egg masses extracted from carrot roots in the infested fields of Kishnagiri district, Tamil Nadu (Figure 1) were allowed to hatch in water, with nine replications. Upon hatching, DNA was extracted from 8-10 second stage juveniles of root-knot nematodes/replication following the method described by Holterman *et al.* (2006) with slight modifications, including intermittent shaking every 15 mins in a dry bath. *M. incognita* species-specific primers, MincF1 (5' AAAAACACGCGATAACAAAAA 3') and MincR1 (5' ATTCAAAACTTGGGGGAAAAA 3') were employed to amplify the target using the extracted DNA (Devran *et al.,* 2018). The PCR cycling conditions were set as follows: an initial denaturation step at 95°C for 3 mins, followed by 35 cycles comprising denaturation at 95°C for 30 s, annealing at 56°C for 30 s, extension at 72°C for 1 min, and finally, a concluding extension step at 72°C for 7 min. The PCR product was visualized on a 1 per cent agarose gel and documented using a gel documentation system. The Posterior Cuticular Pattern (PCP) of females was dissected and examined following the protocol described in Eisenback *et al.* (1980). Following confirmation of their identity as *M. incognita* (Figures 2 a and b), second-stage infective juveniles were

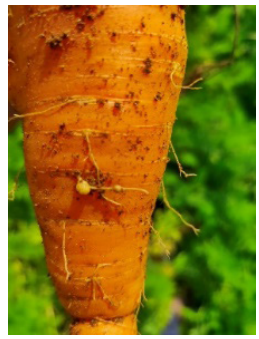
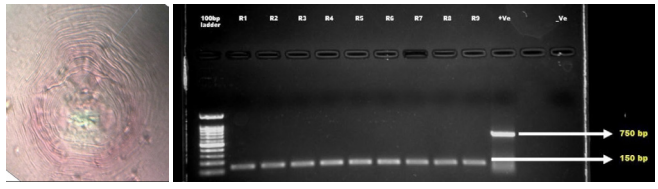


Figure 1. Infested carrot roots collected from the field.



(a) **(b)**

Figure 2. **(a).** Posterior cuticular pattern of RKN isolated from the infected carrot field. **(b).** Molecular confirmation of *M. incognita* (R1-9 = Replications 1 – 9).

inoculated @ 1 J_2 per g of soil into one-month-old carrot plants within a glass house for pure culture maintenance of *M. incognita*. Egg masses collected from this pure culture were utilized for the experiment.

Experimental site and crop husbandry

The pot experiment was conducted in the glass house, the Department of Nematology, Tamil Nadu Agricultural University, Coimbatore during the Kharif season, 2021. The glass house is situated at coordinates N11º00.759', E076º56.223'. Carrot seeds of the Kuroda variety were sown in 5 kg mud pots filled with an autoclaved pot mixture consisting of red soil, sandy soil and FYM in a 2:1:1 ratio. The sowing was done by dibbling, followed by thinning to one seedling per pot at 20 Days After Sowing (DAS). At 45 DAS, newly hatched second-stage juveniles of *M. incognita* were inoculated into the pots @ 1 IJ/g of soil. The plants were irrigated once every two days.

Experimental design

The pot experiment followed a Completely Randomized Design (CRD) comprising five treatments, a chemical check, and an untreated check (Figure 3). Each of these experimental conditions was replicated three times. The treatment details are as follows: T_1 – liquid formulation of *Purpureocilium lilacinum* @ 1 ml/pot/dose, T_2 - liquid formulation of *Pochonia chlamydosporia* @ 1 ml/pot/dose, T_3 - liquid formulation of *Trichoderma viride* @ 1 ml/pot/dose, T_4 - vermiculite formulation of *Rhizophagus intraradices* @ 1 g/pot/dose, T_5

Figure 3. Experimental setup under glasshouse conditions (N11° 00.759', E076° 56.223').

- liquid formulation of *Bacillus subtilis* @1 ml/pot/dose, T_6 – Carbofuran 3G @1 g/pot/dose and T_7 - Untreated check. The first dose was given as soil basal application, whereas the second dose was drenched in soil at 45 DAS.

Parameters studied

Observations on plant growth parameters *viz.,* shoot length, shoot weight, root length, root weight, and nematicidal efficacy facets *viz.,* root-knot index (RKI), IJs population in the soil, number of egg masses, adult female population in the branched and feeder roots were recorded at 120 DAS. The Root Knot Index (RKI) was accorded based on Hartman & Sasser (1- no galls, 2- 1–10 galls, 3- 11–30 galls, 4- 31–75 galls, 5- >75 galls) (1985).

Statistical analysis

The data were subjected to ANOVA and the treatment means were juxtaposed following Duncan's Multiple Range Test (DMRT) using SPSS 16.0 for Windows software (SPSS Inc., Chicago, IL, USA) (Gomez & Gomez, 1984).

RESULTS

Commercially available nematode antagonists were assessed for their proficiency in suppressing the pathogenicity and parasitic success of *M. incognita* in carrots. Furthermore, parameters such as root length, root weight, shoot length, and shoot weight were measured for all treated plants and compared with control plants to evaluate the potential of nematode antagonistic bio-organisms in enhancing the growth and development of carrots. The results indicated significant differences among various treatments in their efficacy against *M. incognita* in carrots.

Shoot length

Greatest per cent of shoot length was recorded in carrot plants applied with the Carbofuran (37.6%),

Table 1. Effect of bio-formulations on growth and yield of carrot *cv.* kuroda

Treatment	Shoot length (cm)	Shoot weight (g)	Root length (cm)	Root weight (g)
T1 - *Purpureocillium lilacinum* @ 1ml/ pot	38.9^{b} (25.4)	19.07^{b} (45.2)	9.66^{b} (49.3)	27.7^{b} (40)
T2 - *Pochonia chlamydosporia* @ 1ml/ pot	45.2^{a} (35.8)	27.88^{a} (62.5)	15.13^{a} (67.5)	36.26^{a} (54.3)
T3 - *Trichoderma viride* @ 1ml/ pot	39.5^{b} (26.5)	27.97^{a} (62.9)	8.83^{b} (44.3)	28.96^{ab} (42.6)
T4 - *Rhizophagus intraradices* @ 1 g/ pot	38.36^{b} (24.2)	17.93^{bc} (41.9)	9.13^{b} (46.3)	27.96^{b} (40.5)
T5 - *Bacillus subtilis* @ 1ml/ pot	44.73^{a} (35.1)	26.38^{ab} (60.7)	14.66^{a} (66.5)	36.13^{a} (54)
T6- Carbofuran @ 1 g/ pot	46.5^{a} (37.6)	23.61^{ab} (56)	12.9^{a} (62)	35.86^{a} (53.7)
T7 – Control	29.03	10.4^{c}	4.9^{c}	16.6^{c}
SEd	1.62	3.96	1.29	3.63
CD 5%	3.47	8.51	2.77	7.79

(SEd - Standard Error of difference between treatment mean Values; CD – Critical Difference). Values are means of three replications. The number in the parentheses indicates a per cent increase over control. Means followed by the same letter in columns are not significantly different at $p<0.05$ according to Duncan's Multiple Range Test (DMRT).

P. chlamydosporia (35.8%) and *B. subtilis* (35.1%), while the least per cent of shoot length was accorded over control in plant with *T. viride* (26.5%), *P. lilacinum* (25.4%) and *R. intraradices* (24.2%) respectively (Table 1 and Figure 4).

Shoot weight

Maximum per cent of shoot weight was noticed in plants applied with *P. chlamydosporia* (62.9%), *B. subtilis* (60.7%), *T. viride* (62.9 %) and Carbofuran (56%) as they were on par with each other. In contrast, the minimum per cent of increased shoot weight was observed in carrot plants applied with *P. lilacinum* (45.2%) and *R. intraradices* (41.9%) (Table 1 and Figure 4).

Root length

The highest per cent of root length was recorded in carrot applied with *P. chlamydosporia* (67.5%), *B. subtilis* (66.5%) and Carbofuran (62%) as they were on par to each other, whereas the least per cent of root length was recorded in carrot applied with *P. lilacinum* (49.3%), *T. viride* (44.3%) and *R. intraradices* (46.3%) (Table 1 and Figure 4).

Root weight

Roots supplied with *P. chlamydosporia* (54.3%), *B. subtilis* (54%) and Carbofuran (53.7%) were on par and recorded a higher per cent of root weight, whereas roots with *P. lilacinum* (40%), *T. viride* (42.6%) and *R. intraradices* (40.5%) were on par to and considered as least effective in increasing root weight (Table 1 and Figure 4).

Number of J_2s/200 cc soil

The higher suppression of soil nematode population was recorded in carrot roots colonized with *P. chlamydosporia* @ 1ml (51.8%) over control. Followed by, a liquid formulation of *B. subtilis* @ 1ml (46.4%) and granular application of Carbofuran @ 1g (45.2%) were on par with each other in reducing the soil nematode population. The liquid formulation of *P. lilacinum* @ 1ml (35.7%) and *T. viride* @ 1ml (31.9%) was ranked third and fourth respectively in lowering the population of second-stage juveniles in soil. *R. intraradices* @ 1g (27.5%) applied carrot roots were noticed to have a greater number of second-stage juveniles in soil than other treatments (Table 2 and Figure 4).

Figure 4. Effect of bio formulations on *M. incognita* infection in carrot (T_1 - *P. lilacinum* @ 1 ml/ pot, T_2 – *P. chlamydosporia* @ 1 ml/ pot, T_3 - *T. viride* @ 1 ml/ pot, T_4 - *Arbuscular mycorrhiza* @ 1 g/ pot, T_5 - *Bacillus subtilis* @1 ml/ pot, T_6 -Carbofuran @1 g/ pot and T_7 - Untreated check).

Number of females/5 g of root

Pochonia chlamydosporia @ 1ml (43.6%) caused a significant reduction in *the* adult female population of root-knot nematode over other treatments including control. Next to that, a liquid formulation of *B. subtilis* @ 1ml (38.9%) and a granular application of Carbofuran @ 1g (38.4%) were on par with each other in considerably reducing the adult female population in the root. This was followed by the liquid formulation of *P. lilacinum* @ 1ml (30.3%) and *T. viride* @ 1ml (30.2%) as they were on par with each other. *R. intraradices* @ 1g (26%) found less in reducing the adult female population of root knot-nematode in roots compared to other treatments (Table 2 and Figure 4).

Number of egg masses/5 g of root

The application of liquid formulation of *P. chlamydosporia* @ 1ml (65.3%) significantly lowered the number of egg masses over the rest of the treatments and control. This was followed by the liquid formulation of *B. subtilis* @ 1ml (56.3%), *P. lilacinum* @ 1ml (54.4%) and granular application of Carbofuran @ 1g (54.4%) as they were on par with each other. The carrot roots were applied with soil drenching of *T. viride* @ 1ml (30%) and *R. intraradices* @ 1g (28.4%) has been observed with a significantly higher number of egg mass compared to other treatments (Table 2 and Figure 4).

Root Knot Index (RKI)

The application of liquid formulation of *P. chlamydosporia* @ 1ml recorded least root gall index (2.3), followed by, plants applied with *B. subtilis* @ 1ml (2.6), Carbofuran @ 1g (2.7), *P. lilacinum* @ 1ml (3.3), *T. viride* @ 1ml (3.6). The number of root galls was more in carrot roots applied with *R. intraradices* @ 1g with an RKI of 4.0 and untreated control plants with an RKI of 4.7 (Table 2 and Figure 4).

DISCUSSION

The results from the *in vivo* experiment against *M. incognita* in carrots indicate that the application of the liquid formulation of *P. chlamydosporia* exhibits significantly higher nematicidal efficacy compared to carbofuran. Meanwhile, the liquid formulation of *B. subtilis* demonstrates comparable effectiveness in nematode control. Additionally, the liquid formulations of *P. lilacinum* and *T. viride* also show the ability to suppress both soil and root nematode population density. All treatments, including Carbofuran, contribute to enhanced root and shoot architecture in carrots compared

Table 2. Effect of bio-formulations on *M. incognita* infection of Carrot *cv.* Kuroda

Treatment	No. of J2s/ 200 cc soil	No. of females/ g of root	No. of egg mass/ g of root	Root Knot Index (RKI)
T1 - *Purpureocillium lilacinum* @ 1ml/ pot	148.00^{c} (35.9)	49.00^{c} (30.3)	8.66^{b} (54.4)	3.33
T2- *Pochonia chlamydosporia* @ 1ml/ pot	111.00^{a} (51.8)	39.66^{a} (43.6)	6.66^{a} (65.3)	2.33
T3 - *Trichoderma viride* @ 1ml/ pot	157.33^{d} (31.7)	49.00^{c} (30.2)	13.33^{d} (30)	3.66
T4 - *Rhizophagus intraradices* @ 1 g/ pot	167.33^{e} (27.5)	52.00^{d} (26)	13.66^{d} (28.4)	4.00
T5 - *Bacillus subtilis* @ 1ml/ pot	123.66^{b} (46.4)	43.00^{b} (38.9)	8.33^{b} (56.3)	2.66
T6 - Carbofuran @ 1 g/ pot	126.33^{b} (45.2)	43.33^{b} (38.4)	8.66^{b} (54.4)	2.77
T7- Control	230.66^{f}	70.33^{e}	19.00e	4.77
SEd	1.7366	1.0690	0.5909	-
CD 5%	3.7251	2.2931	1.2676	-

Values are means of three replications. The numbers in the parentheses indicate a per cent decrease over control. Means followed by the same letter in columns are not significantly different at $p<0.05$ according to Duncan's Multiple Range Test (DMRT).

to the control group. The findings regarding the potential of *P. chlamydosporia* align with similar observations made by other researchers. (Ahmed & Monjil, 2019; Bontempo *et al.,* 2014; Kerry, 2000; Swarnakumari *et al.,* 2020; Viggiano *et al.*, 2014). Moreover, the effectiveness of *B. subtilis* was consistent with findings reported by several other researchers. (Araújo & Marchesi, 2009; Basyony & Abo-Zaid, 2018; Rao *et al*., 2017).

The advantages of employing liquid formulations of bio-agents include a high cell count, extended shelf life, enhanced efficacy, increased resilience against environmental stresses, absence of contamination, and straightforward preparation of the formulation. (Gopalakrishnan *et al.,* 2016; Manikandan *et al.,* 2010). Liquid formulations typically incorporate thickeners and dispersants, contributing to improved stability and preventing sedimentation. Additionally, the presence of wetting agents in liquid formulations helps reduce the surface tension of droplets, enhancing their effectiveness. (Bejarano & Puopolo, 2020). The effectiveness of liquid formulations of bioagents against nematodes has been illustrated in several crops *viz.,* soybean against *Heterodera glycines* (Liu & Chen, 2005), banana against *Helicotylenchus multicintus* (Selvaraj *et al*., 2014) and tomato against *M. incognita* (Song *et al.,* 2016).

In the study, *P. chlamydosporia* was evinced as a better bionematicide than Carbofuran under an *in vivo* environment *viz.,* 14% reduction in root galling, 23% reduction in egg mass formation, 12% reduction in soil root-knot nematode population, 8.5% reduction in root nematode population over chemical control. Concurrently, the incorporation of *P. chlamydosporia var. chlamydosporia* isolate Pc-10 @ 3 kg/ ha increased the total and marketable production of carrot roots by 25.35 and 55.03 % respectively with the reduction in the production of unmarketable roots and the reproduction factor of *M. incognita* by above 50% and it was marked as best treatments over other (Bontempo *et al.*, 2014). Swarnakumari and Kalaiarasan (2017) reported the egg parasitizing behaviour of *P. chlamydosporia* in detail where they explained the dissolution of the egg through that hyphae, mycelium, chalmydospores of *P. chlamydosporia.* The presence of protease and chitinase enzymes would be responsible as virulent factors against nematode (Mi *et al.*, 2010). Aurovertin like metabolites present in *P. chlamydosporia* have shown some similarities to phomalactone, a nematicidal molecule (Niu, 2017). *P. chlamydosporia* can withstand saprophytic in soil (Siddiqui *et al.*, 2009). Some Gramineae and Solanaceae plants have been found to exhibit endophytic behaviour by *Pochonia* species (Lopez-Llorca *et al.*, 2002). The fungus is also a hyperparasite of other fungi and a facultative parasite of worm and mollusc eggs (Zare *et al.*, 2001). Soil with higher sand per cent helps in better colonization of *P. chlamydosporia* (Monteiro *et al.*, 2018).

Similar to our finding Rao *et al.* (2017) found that the *B. subtilis* IIHR BS-2 reduced the egg hatching (94.65%) and increased the juvenile mortality (91.26%) with improved plant growth. *B. subtilis* suppressed the root-knot nematode population by the production of lytic enzymes like chitinase (Antibiosis); production of antibiotic compounds namely cyclic lipopeptides: surfactin, fengycin, iturins, acteriocins, polyketides, bacteriocins; production of volatile organic compounds; enhancement of plant growth and Induced Systemic Resistance (Migunova & Sasanelli, 2021). *B. subtilis* is categorized as plant growth-promoting rhizobacteria and it can activate the host plant response against invading pathogens (Hashem *et al.*, 2019).

CONCLUSION

The study demonstrates that the application of a liquid formulation of *P. chlamydosporia* and *B. subtilis* @ 1ml/ pot/ dose as two doses has the potential to outperform other bio-agents in reducing the parasitic success and pathogenic ability of root-knot nematodes in carrots under *in vivo* conditions. As a result, conducting multi-locational and multi-seasonal field trials would be beneficial to endorse this liquid bio-formulation as an eco-friendly alternative to carbofuran. Additionally, further investigations into the ecology and interactions of bio-agents with other microbiomes in the rhizosphere are needed.

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REFERENCES

Ahmed, S., and Monjil, M. S. 2019. Effect of *Paecilomyces lilacinus* on tomato plants and the management of root knot nematodes. *J Bangladesh Agril Univ,* **17**: 9-13. https://doi.org/10.3329/jbau.v17i1.40657

Araújo, F. F. D., and Marchesi, G. V. P. 2009. Use of *Bacillus subtilis* in the control of root-knot nematode and the growth promotion in tomato. *Cienc Rural,* **39:** 1558-1561. https://doi.org/10.1590/S0103-84782009000500039

Basyony, A. G., and Abo-zaid, G. A. 2018. Biocontrol of the root-knot nematode, *Meloidogyne incognita*, using an eco-friendly formulation from *Bacillus* subtilis, lab. and greenhouse studies. *Egypt J Bìol Pest Control,* **28**: 1-13. https://doi.org/10.1186/s41938-018-0094-4

Bejarano, A., and Puopolo, G. 2020. Bioformulation of microbial biocontrol agents for a sustainable agriculture. How research can stimulate the development of commercial biological control against plant diseases. Springer. https://doi.org/10.1007/978-3-030-53238-3_16

Bontempo, A., Fernandes, R., Lopes, J., Freitas, L., and Lopes, E. 2014. *Pochonia chlamydosporia* controls *Meloidogyne incognita* on carrot. *Australas Plant Pathol,* **43:** 421-424. https://doi.org/10.1007/s13313-014-0283-x

Devran, Z., Polat, I., Mıstanoglu, İ., and Baysal, O. 2018. A novel multiplex PCR tool for simultaneous detection of three root-knot nematodes. *Australas Plant Pathol,* **47**(4): 389–392. https://doi.org/10.1007/s13313-018-0570-z

Directorate of Plant Protection Quarantine and Storage. (n.d.). Ministry of Agriculture and Farmers Welfare, Government of India. ppqs.gov.in

Gomez, K. A., and Gomez, A. A. 1984. *Statistical procedures for agricultural research*, John Wiley and Sons.

Gopalakrishnan, S., Sathya, A., Vijayabharathi, R., and Srinivas, V. 2016. Formulations of plant growth-promoting microbes for field applications. Microbial inoculants in sustainable agricultural productivity. Springer. https://doi.org/10.1007/978-81-322-2644-4_15

Gupta, R. C. 1994. Carbofuran toxicity. *J Toxicol Environ Health - A: Curr Issues,* **43**: 383-418. https://doi.org/10.1080/15287399409531931 PMid:7990167

Hartman, K., and Sasser, J. 1985. Identification of *Meloidogyne* species on the basis of differential host test and perineal-pattern morphology. *An advanced treatise on Meloidogyne,* **2**: 69-77.

Hashem, A., Tabassum, B., and Abd_Allah, E. F. 2019. *Bacillus subtilis*: A plant-growth promoting rhizobacterium that also impacts biotic stress. *Saudi J Biol Sci,* **26**: 1291-1297. https://doi.org/10.1016/j.sjbs.2019.05.004 PMid:31516360 PMCid:PMC6734152

Holterman, M., Van der wurff, A., Van den elsen, S., Van megen, H., Bongers, T., Holovachov, O., and Helder, J. 2006. Phylum-wide analysis of SSU rDNA reveals deep phylogenetic relationships among nematodes and accelerated evolution toward crown clades. *Mol Biol Evol,* **23**: 1792-1800. https://doi.org/10.1093/molbev/msl044 PMid:16790472

INDIASTAT 2022. Socio economic statistical information about India.

Kerry, B. R. 2000. Rhizosphere interactions and the exploitation of microbial agents for the biological control of plant-parasitic nematodes. *Annu Rev Phytopathol,* **38:** 423-441. https://doi.org/10.1146/annurev.phyto.38.1.423 PMid:11701849

Liu, S., and Chen, S. 2005. Efficacy of the fungi *Hirsutella minnesotensis* and *H. rhossiliensis* from liquid culture for control of the soybean cyst nematode Heterodera glycines. *Nematol,* **7:** 149-157. https://doi.org/10.1163/1568541054192153

Lopez-llorca, L. V., Olivares-bernabeu, C., Salinas, J., Jansson, H.-B., and Kolattukudy, P. E. 2002. Pre-penetration events in fungal parasitism of nematode eggs. *Mycol Res,* **106:** 499-506. https://doi.org/10.1017/S0953756202005798

Manikandan, R., Saravanakumar, D., Rajendran, L., Raguchander, T., and Samiyappan, R. 2010. Standardization of liquid formulation of *Pseudomonas fluorescens* Pf1 for its efficacy against Fusarium wilt of tomato. *Biol Control,* **54:** 83-89. https://doi.org/10.1016/j.biocontrol.2010.04.004

Mi, Q., Yang, J., YE, F., Gan, Z., Wu, C., Niu, X., Zou, C., and Zhang, K.-Q. 2010. Cloning and overexpression of *Pochonia chlamydosporia* chitinase gene pcchi44, a potential virulence factor in infection against nematodes. *Process Biochem,* **45:** 810-814. https://doi.org/10.1016/j.procbio.2010.01.022

Migunova, V. D., and Sasanelli, N. 2021. Bacteria as biocontrol tool against phytoparasitic nematodes. *Plants,* **10**: 389. https://doi.org/10.3390/plants10020389 PMid:33670522 PMCid:PMC7922938

Monteiro, T. S. A., Valadares, S. V., De Mello, I. N. K., Moreira, B. C., Kasuya, M. C. M., De araujo, J. V., and De freitas, L. G. 2018. Nematophagus fungi increasing phosphorus uptake and promoting plant growth. *Biol Control,* **123:** 71-75. https://doi.org/10.1016/j.biocontrol.2018.05.003

Niu, X. M. 2017. Secondary metabolites from *Pochonia chlamydosporia* and other species of *Pochonia*. Perspectives in Sustainable Nematode Management Through Pochonia chlamydosporia Applications for Root and Rhizosphere Health. Springer. https://doi.org/10.1007/978-3-319-59224-4_7

Perry, R. N., and Moens, M. 2006. Plant nematology, CABI. https://doi.org/10.1079/9781845930561.0000

Potter, A. S., Foroudi, S., Stamatikos, A., Patil, B. S., and Deyhim, F. 2011. Drinking carrot juice increases total antioxidant status and decreases lipid peroxidation in adults. *Nutr J,* **10**: 1-6. https://doi.org/10.1186/1475-2891-10-96 PMid:21943297 PMCid:PMC3192732

Prasad, G. G., Ravichandra, N. G., Narasimhamurthy, T. N., Kumar, C. P., and Yadahalli, P. 2014. Management of *Meloidogyne incognita* infecting carrot by using bioagents. *J Biopest,* **7:** 144. https://doi.org/10.57182/jbiopestic.7.2.144-150

Rao, M., Kamalnath, M., Umamaheswari, R., Rajinikanth, R., Prabu, P., Priti, K., et al. Gopalakrishnan, C. 2017. *Bacillus subtilis* IIHR BS-2 enriched vermicompost controls root knot nematode and soft rot disease complex in carrot. *Sci Hortic,* **218:** 56-62. https://doi.org/10.1016/j.scienta.2017.01.051

Schroth, M. N., and Hancock, J. G. 1981. Selected topics in biological control. *Annu Rev Microbiol,* **35**: 453-476. https://doi.org/10.1146/annurev.mi.35.100181.002321 PMid:7027904

Seenivasan, N. 2017. 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,* **6:** 1-7. https://doi.org/10.20546/ijcmas.2017.609.446

Selvaraj, S., Ganeshamoorthi, P., Anand, T., Raguchander, T., Seenivasan, N., and Samiyappan, R. 2014. Evaluation of a liquid formulation of *Pseudomonas fluorescens* against *Fusarium oxysporum* f. sp. *cubense* and Helicotylenchus multicinctus in banana plantation. *BioControl,* **59:** 345-355. https://doi.org/10.1007/s10526-014-9569-8

Siddiqui, I., Atkins, S., and Kerry, B. 2009. Relationship between saprotrophic growth in soil of different biotypes of *Pochonia chlamydosporia* and the infection of nematode eggs. *Ann Appl Biol,* **155**: 131-141. https://doi.org/10.1111/j.1744-7348.2009.00328.x

Song, Z., Shen, L., Zhong, Q., Yin, Y., and Wang, Z. 2016. Liquid culture production of microsclerotia of *Purpureocillium lilacinum* for use as bionematicide. *Nematol,* **18**: 719-726. https://doi.org/10.1163/15685411-00002987

Sowmya, D. S., Rao, M. S., Kumar, R. M., Gavaskar, J., and Priti, K. 2012. Bio-management of *Meloidogyne incognita* and *Erwinia carotovora* in carrot (*Daucus carota* L.) using *Pseudomonas putida* and *Paecilomyces lilacinus*. *Nematol Mediterr,* **40:** 189-194.

Swarnakumari, N., and Kalaiarasan, P. 2017. Mechanism of nematode infection by fungal antagonists, *Purpureocillium lilacinum* (Thom) Samson and *Pochonia chlamydosporia* (Goddard) Zare and Gams 2001. *Pest Manag Hort Ecosyst,* **23:** 165-169.

Swarnakumari, N., Sindhu, R., Thiribhuvanamala, G., and Rajaswaminathan, V. 2020. Evaluation of oil dispersion formulation of nematophagus fungus, *Pochonia chlamydosporia* against root-knot nematode, *Meloidogyne incognita* in cucumber. *J Asia Pac Entomol,* **23:** 1283-1287. https://doi.org/10.1016/j.aspen.2020.10.008

Trotter, D. M., Kent, R. A., and Wong, M. P. 1991. Aquatic fate and effect of carbofuran. *Crit Rev Environ Sci Technol,* **21:** 137-176. https://doi.org/10.1080/10643389109388412

Viggiano, J. R., De Freitas, L. G., and Lopes, E. A. 2014. Use of Pochonia chlamydosporia to control *Meloidogyne javanica* in cucumber. *Biol Control,* **69**: 72-77. https://doi.org/10.1016/j.biocontrol.2013.11.004

Viljoen, J. J., Labuschagne, N., Fourie, H., and Sikora, R. A. 2019. Biological control of the root-knot nematode *Meloidogyne incognita* on tomatoes and carrots by plant growth-promoting rhizobacteria. *Trop Plant Pathol,* **44:** 284-291. https://doi.org/10.1007/s40858-019-00283-2

Wesly, J. L., Kalaiarasan, P., Devrajan, K., Shanthi, A., Rajesh, S., and Elayarajan, M., 2021. Influence of edaphic factors on root-knot nematodes, *Meloidogyne* sp. infesting carrot (*Daucus carota*) grown in Tamil Nadu, India. *Indian J Nemato*, **51**(2): 166-174. https://doi.org/10.5958/0974-4444.2021.00024.X

Zare, R., Gams, W., and Evans, H. 2001. A revision of *Verticillium* section Prostrata. V. The genus *Pochonia*, with notes on Rotiferophthora. *Nova Hedwig,* **73:** 51-86. https://doi.org/10.1127/nova.hedwigia/73/2001/51