Harnessing the biocontrol potential of entomopathogenic nematode, *Steinernema* species for the management of root-knot nematode, *Meloidogyne incognita* in tomato (*Solanum lycopersicum* L)

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ABSTRACT

Root-knot nematodes (*Meloidogyne incognita*) are major constraints to tomato production, causing severe yield losses worldwide. The present study evaluated the biocontrol potential of four entomopathogenic nematode (EPN) species: *Steinernema carpocapsae*, *S glasseri*, *S siamkayai* and *S feltiae* against *M incognita* under in vitro and pot culture conditions. In in vitro assays, all *Steinernema* species significantly inhibited egg hatching, with *S carpocapsae* and *S siamkayai* showing the highest suppression, comparable to carbofuran. Pot culture experiments revealed that EPN treatments significantly improved plant growth and yield attributes while reducing nematode population, gall number and gall index compared to the untreated control. Among the EPNs, *S carpocapsae* was the most effective, followed by *S siamkayai* and *S feltiae*. The results demonstrate the potential of *Steinernema* species, particularly *S carpocapsae*, as eco-friendly alternatives to chemical nematicides for sustainable management of root-knot nematodes in tomato.

Keywords: Entomopathogenic nematodes; *Steinernema* spp; Root-knot nematode; *Meloidogyne incognita*; Tomato; Biological control; Sustainable agriculture

INTRODUCTION

Root-knot nematodes (*Meloidogyne* spp), one of the most damaging plant-parasitic nematodes, cause significant yield losses in a wide range of agricultural crops worldwide. They are major biotic stress causing agents for several crops globally (Khan et al 2023). Among them, *Meloidogyne incognita* is the most prevalent and economically significant species, particularly in vegetable crops such as tomato (*Solanum lycopersicum* L). This nematode is the major limiting factor in tomato production in many regions of the world (Abrar et al 2020). Tomato is one of the most popular and widely consumed vegetable crops all over the world (Sabarinath et al 2020) cultivated extensively for its nutritional and economic value; however, its productivity is severely constrained by root-knot nematode infestation. The formation of characteristic root galls by root-knot nematode disrupts water and nutrient uptake, leading to stunted growth, reduced yield and significant economic losses in plants. In India, *Meloidogyne* species cause an estimated 23-38 per cent reduction in tomato yields, resulting in economic losses of approximately 6,000-7,000 million rupees annually (Kumar et al 2020a, 2020b).

Chemical control is still considered as key approach for nematode management. Due to high rate of nematode suppression, chemicals are preferred by the farming community (Jabbar et al 2019).

Traditionally, nematode management has relied on chemical nematicides. Although effective, their repeated and indiscriminate use has led to nematode resistance, reduced efficacy and serious concerns related to environmental pollution and risks to human health and non-target organisms (Bobokulovna et al 2024). These limitations highlight the urgent need for eco-friendly, sustainable and biologically-based alternatives for nematode management.

Entomopathogenic nematodes (EPNs) have been used to manage different nematode species both in greenhouse and field conditions (Krithika et al 2024). Two most important families of EPNs are Steinernematidae and Heterorhabditidae (Koppenhofer and Kaya 2001). The infective juveniles (IJs) of genus *Steinernema* are associated with their symbiotic bacteria, *Xenorhabdus* spp (Kumar et al 2022) which produce a wide array of bioactive metabolites, including toxins, enzymes, antibiotics and secondary metabolites with nematicidal properties. These compounds can adversely affect the survival, reproduction and development of plant parasitic nematodes by disrupting egg masses, inhibiting egg hatching and reducing juvenile viability. In addition, interactions between EPNs, their symbiotic bacteria and soil microorganisms, may directly affect plant defense mechanisms and pathogen suppression (Mikaia 2025). They may compete with plant parasitic nematodes for ecological niches, thereby, contributing to nematode suppression through multiple mechanisms.

It has been demonstrated that EPNs can effectively control *Meloidogyne* spp (Gazit et al 2000, Mokrini et al 2020). Applying EPN infective juveniles from various strains has significantly reduced *Meloidogyne* spp in terms of egg masses (Bal and Grewal 2015), the number of eggs (Langford et al 2014) and second-stage juvenile infectivity within the root matrix (Benseddik 2020).

Therefore, systematic evaluation of different *Steinernema* species under controlled and semi-controlled conditions is essential to identify the most effective species for biological control of *M incognita*.

In this context, the present study was undertaken to evaluate the antagonistic potential of different *Steinernema* species against *M incognita* under in vitro and pot culture conditions. The specific objectives were to assess the effect of *Steinernema* spp on egg hatching of *M incognita*, tomato plant growth and yield attributes, nematode population dynamics and gall formation. The results of this study are expected to provide scientific evidence supporting the use of entomopathogenic nematodes as eco-friendly alternatives to chemical nematicides and to contribute to the development of sustainable nematode management strategies in tomato cultivation.

MATERIAL and METHODS

The experiments were conducted under laboratory and pot culture conditions in the Department of Nematology, Dr MS Swaminathan Agricultural College and Research Institute, Thanjavur, Tamil Nadu in the year 2025. *M incognita* was maintained in greenhouse conditions on tomato (*S lycopersicum* L cv PKM-1). *Steinernema carpocapsae*, *S glasseri*, *S siamkayai* and *S feltiae* were obtained from the culture collection maintained at the Department of Nematology, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu and multiplied using *Corcyra cephalonica* within the technique of insect baiting.

Maintenance of pure culture of root-knot nematode, *M incognita*: Pure culture of *M incognita* was established by inoculating second stage juveniles (J2) into tomato seedlings grown in sterilized potting mixture. Second stage juveniles were collected from freshly collected egg masses from infected roots using sterile forceps.

Mass multiplication of *Steinernema* species: The *Steinernema* species were multiplied on final instar larvae of *Corcyra cephalonica* using White trap method. Infective juveniles (J3) emerging from cadavers were collected, quantified using counting dish and stored at 5°C until use. The concentration of IJs was adjusted to required levels using distilled water.

Experimental design and treatments: The effect of *Steinernema* species on egg hatching of *M incognita* was evaluated under in vitro conditions by completely randomized design with three replications. Five uniform size egg masses of *M incognita* were surface-sterilized and transferred into sterile Petri dishes (4 cm diameter) containing distilled water. Each treatment received 3 ml of *Steinernema* infective juveniles @ 500 IJ per ml. Carbofuran 3G was applied

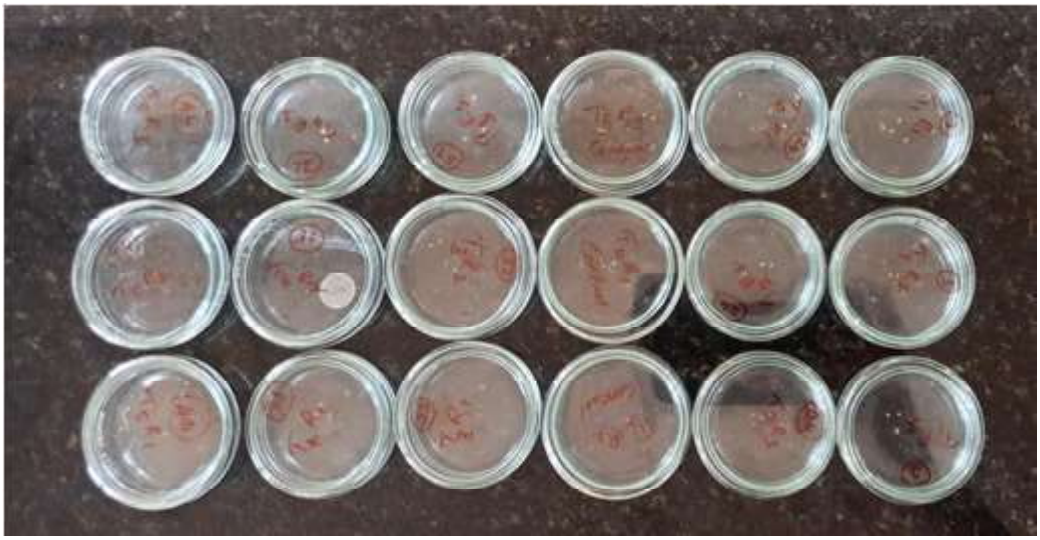


Plate 1. Effect of entomopathogenic nematodes (EPNs) on root-knot nematode eggs under in vitro conditions

@ 0.5 g per plate, while the control received only distilled water (Plate 1).

Petri dishes were incubated at room temperature (25 ± 2°C). The number of hatched juveniles was recorded daily for seven days under stereomicroscope. Per cent egg hatching inhibition was calculated using the formula:

$$\text{Per cent egg hatching inhibition} = \frac{C - T}{C} \times 100$$

where C = Number of juveniles hatched in control, T = Number of juveniles hatched in treatment

Pot culture experiment

It was conducted in a completely randomized design with three replications. Two kg capacity earthen pots were filled with sterilized potting mixture (soil:sand:FYM, 2:1:1). Tomato seedlings (cv PKM-1) were transplanted into pots. After one month, each pot was inoculated with 2,000 second stage juveniles of *M incognita*. After 48 hours, various treatments were applied around the root zone. The treatments were T_1 {(*S carpocapsae* (2×10^6 IJ/pot)}, T_2 {(*S glasseri* (2×10^6 IJ/pot)}, T_3 {(*S siamkayai* (2×10^6 IJ/pot)}, T_4 {(*S feltiae* (2×10^6 IJ/pot)}, T_5 {(Carbofuran 3G (3 g/plant)} and T_6 (Control) (Plate 2).

The experiments were terminated 60 days after inoculation and the plant growth parameters were recorded. The final nematode population from soil was assessed by Cobb's sieving and decanting method (Cobb 1918) followed by modified Baermann's funnel technique (Christie and Perry 1951). After harvest, number of galls per root system and gall index were rated using 0-5 scale (Taylor and Sasser 1978).

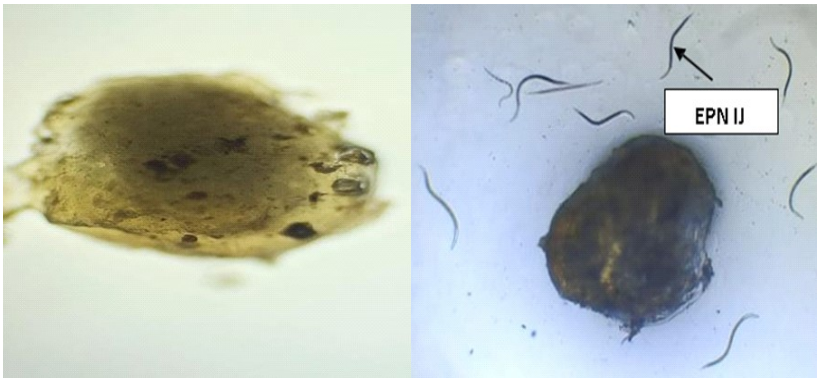
Statistical analysis: The data were subjected to analysis of variance (ANOVA) using appropriate statistical design. Percentage data were subjected to arc sine transformation before analysis.

RESULTS

Effect of entomopathogenic nematode, *Steinernema* species on egg hatching inhibition of root-knot nematode eggs

Biocontrol potential of different species of *Steinernema* on hatching of *M incognita* eggs was made under in vitro condition. The results revealed that all treatments significantly inhibited the hatching of root-knot nematode egg masses (Table 1). On the other hand, enhanced egg hatching was found in untreated control (Plate 3). Among the treatments, carbofuran 3G @ 3 g per plant (T_5) recorded the highest inhibition of egg hatching throughout the experimental period, with values ranging from 59.0 per cent on the 1st day to 46.3 per cent on the 7th day. Next best treatments were *S carpocapsae* (T_1) and *S siamkayai* (T_3), which resulted in 57.0 and 54.3 per cent inhibition respectively on 1st day and 55.3 and 52.3 per cent inhibition respectively on 2nd day. On the 3rd day, *S carpocapsae* (T_1) and *S siamkayai* (T_3) resulted in 52.3 and 51.0 per cent and on 4th day 50.7 and 50.3 inhibition respectively and were at par. However on 5th to 7th day, *S carpocapsae* (T_1) exhibited higher inhibition of 49.7, 44.0 and 41.7 per cent respectively, followed by

Plate 2. Pot culture study of entomopathogenic nematodes and carbofuran application on root-knot nematode-infected tomato plants



Healthy egg mass EPN infected egg mass

Plate 3. Healthy and entomopathogenic nematodes-infected egg mass of root-knot nematode

S siamkayai (T_3) resulting in 46.7, 42.3 and 39.3 per cent inhibition respectively. Thus in overall, *S carpocapsae* (T_1) and *S siamkayai* (T_3) proved superior to *S glasseri* (T_2) and *S feltiae* (T_4) throughout the experimentation. The control treatment (T_6) exhibited the least inhibition throughout the study period, confirming the effectiveness of both chemical and biological treatments in suppressing *M incognita* egg hatching.

Effect of *Steinernema* species on plant growth and *M incognita* population

Plant growth parameters: The results clearly indicate that all treatments significantly influenced plant growth parameters and nematode population compared to the untreated control (Table 2). Among the treatments, carbofuran 3G and *S carpocapsae* recorded the maximum shoot length of 62.12 and 60.95 cm with increase of 24.48 and 22.14 per cent respectively over control and were at par. Maximum root length (27.32 cm) was recorded in carbofuran 3G treatment representing increase of 34.44 per cent, followed by *S carpocapsae* (25.85 cm) and *S siamkayai* (24.95 cm) with increase of 27.21 and 22.78 per cent respectively over control, the two treatments being at par. The treatments, carbofuran 3G (18.77 g), *S feltiae* (17.82 g) and *S carpocapsae* (17.77 g) resulted in maximum shoot weight with increase of 28.82, 22.30 and 21.96 per cent respectively over control and were at par. Maximum root weight (11.75 g), with 65.02 per cent increase over control was observed in carbofuran 3G followed by *S carpocapsae* having root weight of 10.52 g, with 47.75 per cent increase over control. Number of fruits was maximum (4.75/plant) in case of carbofuran 3G (72.72% increase over control) followed by *S carpocapsae*, *S siamkayai, S feltiae* and *S glasseri* having 4.10, 4.00, 3.90 and 3.75 fruits per plant with 49.09, 45.45, 41.81 and 36.36 per cent increase respectively over control, all four treatments being at par. Thus chemical and biological treatments positively influenced reproductive yield.

Nematode population: A substantial reduction in nematode population was observed in all treated plants. Carbofuran 3G (7.75 J2/100 g soil) and *S carpocapsae*

Table 1. Effect of *Steinernema* species on inhibition of root-knot nematode egg hatching

Treatment	Per cent inhibition of egg hatching						
	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day
Steinernema carpocapsae (T_1)	57.0 (49.00)	55.3 (48.10)	52.3 (46.40)	50.7 (45.30)	49.7 (44.70)	44.0 (41.60)	41.7 (40.20)
S glasseri (T_2)	47.0 (43.30)	42.0 (40.40)	39.0 (38.70)	36.3 (37.10)	39.5 (39.00)	32.4 (34.70)	31.0 (33.80)
S siamkayai (T_3)	54.3 (47.60)	52.3 (46.40)	51.0 (45.50)	50.3 (45.10)	46.7 (43.10)	42.3 (40.60)	39.3 (38.90)
S feltiae (T_4)	47.3 (43.50)	43.7 (41.40)	42.0 (40.40)	41.0 (39.80)	39.0 (38.70)	36.3 (37.10)	33.3 (35.20)
Carbofuran 3G (T_5)	59.0 (50.30)	59.7 (50.80)	57.7 (49.40)	57.3 (49.20)	54.7 (47.90)	48.7 (44.10)	46.3 (42.80)
Control (T_6)	41.3 (40.00)	40.0 (39.20)	37.7 (37.90)	35.0 (36.20)	35.3 (36.40)	32.3 (34.60)	29.7 (33.00)
Mean	50.21	47.95	45.71	44.22	43.31	38.85	36.28
SEd(±)	2.70	2.56	2.45	2.36	2.32	2.12	2.01
$CD_{0.05}$	1.21	1.15	1.10	1.06	1.04	0.95	0.90

Figures in parentheses are arcsine transformed values

Table 2. Effect of *Steinernema* species on plant growth and root-knot nematode population in tomato (PKM 1)

Treatment	Plant growth parameter				Nematode population			
	Shoot length (cm)	Root length (cm)	Shoot weight (g)	Root weight (g)	Number of fruits/plant	Juvenile population/ 100 g soil	Number of galls/plant	Gall index
Steinernema carpocapsae (2×10^6IJ/pot)	60.95 (22.14)	25.85 (27.21)	17.77 (21.96)	10.52 (47.75)	4.10 (49.09)	9.00 (–78.94)	8.75 (–73.48)	2
S glasseri (2×10^6IJ/pot)	52.02 (4.24)	22.55 (10.97)	15.15 (3.98)	8.42 (18.25)	3.75 (36.36)	11.00 (–74.26)	11.50 (–65.15)	3
S siamkayai (2×10^6IJ/pot)	54.42 (9.05)	24.95 (22.78)	15.82 (8.57)	8.55 (20.08)	4.00 (45.45)	10.80 (–74.73)	11.25 (–65.90)	3
S feltiae (2×10^6IJ/pot)	53.42 (7.05)	22.95 (12.94)	17.82 (22.30)	9.55 (34.12)	3.90 (41.81)	10.80 (–74.73)	9.30 (–71.81)	2
Carbofuran 3G (3 g/pot)	62.12 (24.48)	27.32 (34.44)	18.77 (28.82)	11.75 (65.02)	4.75 (72.72)	7.75 (–81.87)	8.00 (–75.75)	2
Control	49.90	20.32	14.57	7.12	2.75	42.75	33.00	4
SEd(±)	1.18	0.62	0.45	0.34	0.23	1.12	0.96	0.30
$CD_{0.05}$	2.63	1.38	1.00	0.76	0.51	2.50	2.14	0.67

Figures in parentheses indicate per cent increase or decrease over control

(9.00 J2/100 g soil) showed lowest juvenile population and were at par, corresponding to 81.87 and 78.94 per cent reduction respectively over control. *S siamkayai* (10.80 J2/100 g soil), *S feltiae* (10.80 J2/100 g soil) and *S glasseri* (11.00 J2/100 g soil) lead to 74.73, 74.73 and 74.26 per cent decrease respectively over control and were at par. Carbofuran (8.00 galls/plant), *S carpocapsae* (8.75 galls/plant) and *S feltiae* (9.30 galls/plant) showed the lowest gall number with 75.75, 73.48 and 65.90 per cent decrease respectively over control and were at par. The gall index was also reduced from 4 in the control to 2 in Carbofuran 3G, *S carpocapsae*

and *S feltiae* and to 3 in *S glasseri* and *S siamkayai* treated plants, indicating the efficacy of *Steinernema* towards suppression of *M incognita* infection.

DISCUSSION

The superior performance of carbofuran may be attributed to its systemic nematicidal action, which directly affects egg viability and juvenile emergence. However, the promising inhibitory effect was observed in EPN treatments, particularly *S carpocapsae* and *S siamkayai*, suggesting their potential as eco-friendly alternatives to chemical nematicides. As EPN can enter tomato roots and release symbiotic bacteria that produce allelochemicals that are lethal or antagonistic to *M incognita*, EPNs may decrease the populations of plant parasitic nematodes (PPNs) (Grewal et al 1999).

Application of *S feltiae* in tomato two weeks after the release of *M hapla* eggs and juveniles inhibited the penetration of PPNs in tomato roots (Perez and Lewis 2004). Numerous factors may influence EPNs' suppressive effects on PPNs. For instances, attraction of *Steinernema* species towards tomato roots and suppression may be due to competition between two nematode groups for space, increased density of predators resulting from the application of nematode biomass to the soil (Ishibashi and Kondo 1986), production of allelochemicals by EPNs' symbiotic bacteria complex (Hu et al 1999, Lewis et al 2001). The peroxidase and catalase enzymes, which cause systemic resistance in plants, were induced by *Steinernema* infective juveniles and their symbiotic bacteria. *Steinernema* species are known to produce symbiotic bacteria *Xenorhabdus* spp that release a range of bioactive metabolites, including toxins, enzymes and antibiotics, which can adversely affect nematode eggs and juveniles. It may interfere with egg mass integrity and reduce embryonic development through enzymatic degradation and competitive interactions in the rhizosphere (Grewal et al 1999).

The present findings corroborate earlier reports on the antagonistic interaction between EPNs and root-knot nematodes and highlight the potential of *S carpocapsae* and *S siamkayai* as effective biological control agents. These results indicate that EPNs could be integrated into sustainable nematode management strategies, reducing reliance on chemical nematicides and contributing to environmentally safe crop protection.

CONCLUSION

The present investigations clearly demonstrated that entomopathogenic nematodes possess significant antagonistic activity against *Meloidogyne incognita* in tomato. Both in vitro and pot culture studies confirmed that *Steinernema* species effectively inhibited egg hatching, reduced nematode population density and gall formation and enhanced plant growth and yield parameters. Among the tested species, *S carpocapsae* exhibited the highest efficacy, performing comparably to carbofuran, followed by *S siamkayai* and *S feltiae*. These findings highlight the potential of *Steinernema* species as environmentally safe and sustainable alternatives to chemical nematicides. Integration of EPNs into nematode management programmes could reduce chemical dependence and contribute to eco-friendly tomato production systems.

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