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Sustainable management of *Meloidogyne incognita* in tomato using crude extracts from *Xenorhabdus nematophila*

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Abstract
Root knot nematodes, particularly *Meloidogyne incognita*, pose a significant threat to global agriculture, causing extensive damage to various crops and leading to substantial economic losses. Chemical nematicides have raised environmental concerns, alternative strategies that are eco-friendly and sustainable are the need for the hour. This study aimed to investigate the potential of crude extract derived from *Xenorhabdus nematophila* (MK977604) a symbiotic bacterium associated with entomopathogenic nematodes *Steinernema carpocapsae* (MK977607), for the management of *M. incognita*. Crude extracts at *X. nematophila* at 1.5% concentration significantly inhibit egg mass hatching (no hatching) and juvenile mortality (95.10). The present research revealed that *X. nematophila* exhibits nematicidal properties and can suppress the population of root knot nematodes at lower concentration under *in vitro* study.

Keywords: Entomopathogenic nematode (EPN), *Galleria mellonella*, *Meloidogyne incognita*, crude extract, *Xenorhabdus nematophila*

Introduction
Entomopathogenic nematodes (EPNs) belong to the families Heterorhabditidae and Steinernematidae are obligate insect parasites that spend part of their life cycles in hosts (Shapiro-Ilan and Dolinski 2015) [13]. EPNs are used against a variety of pest, insects, nematodes and mites (Van Zyl and Malan 2015) [15]. The genera *Steinernema* and *Heterorhabditis* form mutualistic associations with bacteria belonging to the genera *Xenorhabdus* and *Photorhabdus*, respectively. EPNs have been exploited as biological control agents since the last half of the 20th century, much research remains to be done to understand how these organisms function in agricultural and other ecosystems (Hatting *et al.*, 2017) [7]. IJs locate, choose, and infect a host either through a natural opening or by penetrating its cuticle.

Material and Methods
Maintenance of insect culture
Galleria mellonella, were reared in the laboratory using an artificial diet specifically designed for their nutritional needs (Eischen and Dietz 1990) [4]. The late instars of *G. mellonella* larvae were systematically collected and utilized for the purpose of rearing EPN (Devi and Gitanjali 2021) [3].

Maintenance of Entomopathogenic nematode
EPN Steinernema carpocapsae (MK977607) was obtained from the Department of Nematology, Tamil Nadu Agricultural University, Coimbatore and it was used in this study. The nematode was maintained on the greater wax moth *G. mellonella* (Pyralidae: Lepidoptera) throughout the study period (Kulkarni *et al.*, 2017) [19].

Maintenance of Root-knot nematode culture
M. incognita, the root-knot nematode (race 3) used in the study, was being maintained at the Department of Nematology, Tamil Nadu Agricultural University, Coimbatore. The nematode was being kept in PKM 1 tomato plants under glasshouse conditions and was utilized in the study as and when required (Srivastava and Chaubey 2020) [14].

Isolation of Symbiotic Bacteria

Xenorhabdus nematophila (MK977604) symbiotic bacteria from *Steinernema carpocapsae* (MK977607) was isolated from the hemolymph of *G. mellonella* which infested with nematode two days earlier to extraction. The hemolymph was streaked into NBTA medium (Akhurst 1980) [2]. The agar plates were maintained at 28 °C overnight. Subsequently individual colonies of *X. nematophila* were selected based on color characters using a sterile inoculating loop and transferred to NBTA plates until future use.

Preparation of crude extract

Purified bacterial colonies obtained from NBTA plates were introduced into NB broth and positioned on a shaker at room temperature, operating at 40 rpm, for a span of 72 hours (Abebew, Sayedain *et al.,* 2022) [1]. Subsequently, the bacterial cells underwent separation through centrifugation at 13,000 rpm for a duration of 15 minutes using 50 ml centrifuge tubes. The resulting supernatant was then filtered through a Millipore filter with a pore size of 0.22 micrometers (µm) to ensure the exclusion of bacterial cells from the filtrate. This resulting solution is referred to as the stock solution, which was subsequently diluted to various concentrations, namely 0.5%, 1%, and 1.5%, utilizing sterile distilled water. The distinct dilutions as well as the stock solution were stored at 4 °C until future study.

Effect of Crude extract on eggs of *M. incognita*

An *in vitro* study was carried out utilizing distinct concentrations (0.25%, 0.5%, and 1%) of crude extract, in conjunction with egg masses of *M. incognita*. Additionally, a control consisting of eggs in 1000 (µl) of tap water was used. This experimental setup was replicated four times, and the number of hatched juveniles was documented at intervals of 12, 24, 48, and 72 hours (Srivastava and Chaubey 2020) [14]. The percentage of egg hatching was measured for each treatment.

Effect of Crude extract on juveniles of *M. incognita*

An *in vitro* study, with various concentrations (0.25%, 0.5%, 1% and 1.5%) of crude extract along with 100 recently hatched second-stage juveniles (J2s) of *M. incognita* along with control in tap water. This experiments was replicated four times, and the number of lifeless juveniles was documented at 12, 24, 48, and 72-hour intervals (Srivastava and Chaubey 2020) [14]. To confirm mortality, infective juveniles with no movement were delicately probed and placed in water for an hour. Juveniles that remained motionless were deemed departed, and the percentage of mortality was recorded for each treatment.

Penetration study

Tomato seeds treated with both distilled water and crude extracts from *Xenorhabdus* bacteria were placed in Petri plate containing phytogel. Five days after germination, nematode suspension contain 500 IJs per plants was introduced to a corner of the Petri plate, and the nematodes movement were recorded for 5 days. (Williamson *et al.,* 2017) [16].

Results and Discussion

Effect of Crude extract on eggs of *M. incognita*

The effect of different concentrations of *X. nematophila's* crude extracts on the egg hatching of *M. incognita* was investigated *in vitro*, revealed no egg hatching was observed at 1.5% concentration sustaining until the 72-hour followed by 1% with hatching of 12.93% until 72 h. Highest hatching of 55.81% was observed at 0.25% at 72 h (Table 1.) A significant difference was observed in each treatment. Similar hatching inhibition and juvenile paralysis was observed at dosages of 10^6 and 10^7 cells ml-1 by Samaliev *et al.,* (2000) [12] & Fatemeh *et al.,* (2019) [5] in *M. javanica*.

Effect of CFC on juvenile mortality of *M. incognita:*

The efficency of various concentrations of crude extract of *X. nematophila* on juvenile mortality of *M. incognita* under *in vitro* revealed at 1% and 0.5% significantly highest mortality of 95.10% and 94.80% was recorded after 72 h. At 0.25% concentration 85.90% mortality was achieved within 12 h of incubation (Table 2) (Grewal, Lewis *et al*. 1999) [6] (Pulavarty, Horgan *et al*. 2020) [11] & (Maru, Siddiqui *et al.* 2013) [10]. The study's outcomes align with previous research indicating that the symbiotic bacterium *X. nematophila* sourced from *S. carpocapsae* where effectively inhibits *M. javanica* in tomato plants at concentrations of 10^6 and 10^7 cells ml^{-1}. The results was in accordance with the works of Samaliev, Andreoglou *et al.,* (2000) [12], Srivastava and Chaubey (2020) [14], and Hu, Li *et al.,* (1999) [8].

Effect of crude extract on nematode movement

The effect of crude extract on nematode movement revealed that tomato seeds treated with crude extract resulted in the inhibition of nematode movement. This may be due to the crude extract affected the nematodes' sensory organ and affects the response to the environment, So the nematode exhibit erratic movement (Fig.1b). Tomato seeds treated with distilled water exhibited greater nematode movement towards it (Fig.1a). The findings of this study were consistent with Williamson *et al.,* (2017) [16].

Table 1: Effect crude extract filtrateof *X. nematophila* on eggs of *M. incognita*

Treatments	% of Egg hatching			
	12 hrs	24 hrs	48 hrs	72 hrs
T_1-CCF @ 0.25% concentration	28.83 (32.74)	39.95 (39.14)	55.81 (48.17)	55.81 (48.17)
T_2-CCF @ 0.5% concentration	18.19 (25.09)	20.90 (27.04)	26.32 (30.95)	26.32 (30.95)
T_3-CCF @ 1% concentration	7.91 (16.50)	9.63 (17.93)	12.93 (21.38)	12.93 (21.38)
T_4-CCF @ 1.5% concentration	0 (1.43)	0 (1.43)	0 (1.43)	0 (1.43)
T5- Control	60.87 (51.36)	75.32 (60.50)	84.56 (67.08)	98.28 (82.13)
SE(d)	0.383	0.549	0.682	1.494
CD	0.835	1.201	1.472	3.250

*means of four replications; The values in the parentheses are arcsine transformed value.

Table 2: Effect Crude extract of *X. nematophila* on per cent juvenile mortality of *M. incognita*

Treatments	% juvenile mortality of *M. incognita*			
	12 h	24 h	48 h	72 h
T_1-CCF @ 0.5% concentration	35.85 (36.76)	57.01 (48.80)	75.60 (60.53)	85.90 (68.07)
T_2-CCF @ 1% concentration	48.93 (44.40)	65.90 (54.37)	81.40 (64.42)	94.80 (76.44)
T_3-CCF @ 1.5% concentration	56.26 (48.49)	76.17 (61,04)	92.40 (74.22)	95.10 (77.86)
T4- Control	15.70 (23.15)	23.58 (29.21)	39.33 (38.77)	69.43 (56.41)
SE(d)	0.334	0.750	0.655	0.941
CD	0.755	1.698	1.544	2.131

*means of four replications; values in the parentheses are arcsine transformed value.

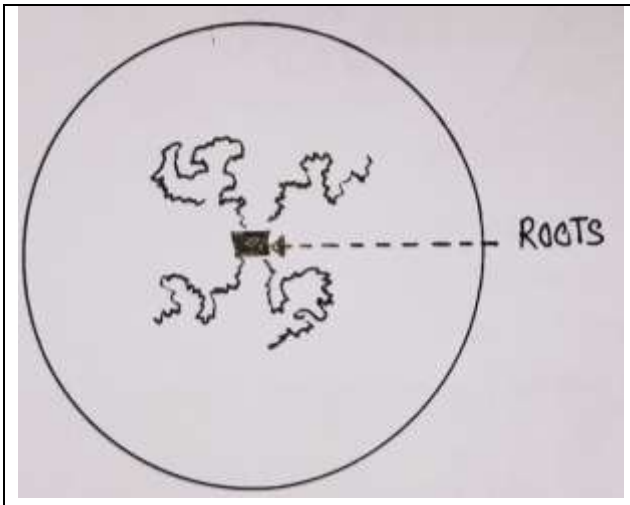

Fig 1a: Nematode attracted towards the roots

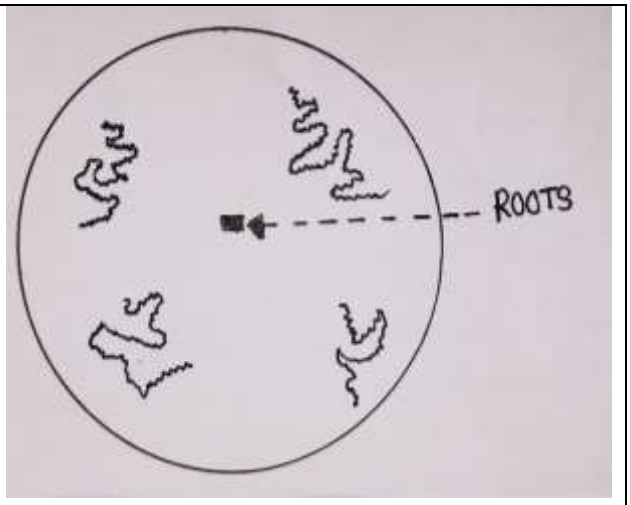

Fig 1b: Nematode deflected away from roots

Fig 1: Effect of crude extract on nematode movement in pluronic gel

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