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RESEARCH

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Integration of some organic amendments and the predatory nematode, *Fictor composticola*, for the management of *Meloidogyne incognita* in cucumber

Harjot Singh Sidhu* and Rambir Singh Kanwar

Abstract

Background: Organic amendments are well known for influencing soil nematode community structure, diversities, and activities. Most of the previous studies focused on effects of organic amendments on plant-parasitic nematodes, but only a few investigated the effect of combination of biocontrol agents and organic amendments on soil nematodes.

Main body: Different organic amendments (neem cake, poultry manure, and neem leaves) were combined with the predatory nematode, *Fictor composticola*, for the control of root-knot nematode, *Meloidogyne incognita*, in cucumber. Organic amendments were mixed in pots containing 1 kg soil, 15 days before sowing. Cucumber (cv. CCH-1) plants grown in these pots were inoculated by 2000 J_2 of *M. incognita* and 400 *F. composticola* per pot, after 1 week of germination. *Fictor composticola* in combination with chicken manure + neem cake + neem leaves was found the best mixture for reducing the number of galls, egg masses, and final root-knot nematode population. The predator's population reached the maximum in this treatment. All combinations of organic amendments in the presence of *F. composticola* were found significantly superior over inoculated check in reducing nematode's population and in improving plant growth over the un-inoculated check.

Conclusions: Data indicated the suitability of using the predatory nematode, *F. composticola*, and organic amendments for potential use in sustainable nematode management strategies.

Keywords: Biological control, Predatory nematode, *Fictor composticola*, *Meloidogyne incognita*, Organic amendment

Key messages

- In context to environmental safety, the application of organic amendments is still the primary option to manage phytonematodes. In this study, we demonstrated that predatory nematode, *Fictor composticola*, which was highly effective against *M. incognita* in earlier studies, was combined with organic amendments has significantly enhanced cucumber growth in pot trials.
- To our knowledge, this is the first report of the diplogasterid predator in combination with organic amendments against *M. incognita* in cucumber production.
- In a cucumber pothouse, the predator population was maximum in combination of three amendments (chicken manure + neem cake + neem leaves) and their incorporation showed some growth promotion effect in cucumber.

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Background

Cucumber is a leading vegetable crop planted in greenhouses (Mao et al. 2016). The crop is attacked by several nematodes, which have become a serious threat, particularly under protected cultivation (Patil et al. 2017). Root-knot nematodes (*Meloidogyne incognita* and *M. javanica*) are the prominent problem that has led to failure/closure of many polyhouses in India. Although considerable work has been carried out during the last few decades on the use of organic amendments for reducing the plant-parasitic nematodes (Singh and Sitaramaiah 1973; Rodriguez-Kabana 1986), but almost no work has been done to recognize the effect of organic amendment over predatory nematodes of Order Diplogasterida, which are known to feed upon the plant-parasitic nematodes and may serve as biological control agents. *Fictor composticola*, a predatory nematode that has shown a potential to control mycophagous and plant-parasitic nematodes (Kanwar et al. 2009; Bajaj and Kanwar 2015). The present work was undertaken to study the management of *M. incognita* by integrating *F. composticola* with some organic amendments, found effective in preliminary trials (Sidhu and Kanwar 2019).

Materials and methods

Location

The experiment was carried out under the screen house conditions in the Department of Nematology, Chaudhary Charan Singh Haryana Agricultural University (CCS HAU), Hisar, Haryana, India during May-June 2019.

Nematode inoculum

Pure culture of *M. incognita* was maintained on brinjal plants (cv. BR 112) in pots. The culture of *F. composticola* was maintained on 1% water agar medium in Petri plates in a BOD incubator at 25 ± 2 °C, using *M. incognita* juveniles as prey. The culture was used for experimentation. For inoculation in pots, the predatory nematodes from culture plates were extracted in sterile water inside a beaker. The predatory nematodes in the beaker were counted and used for the inoculation as per the requirement of the experiment.

Organic amendments

Fresh leaves of neem (*Azadirachta indica*) were collected and chopped by scissors in small pieces and used freshly during the experiment. Locally available neem seed cake was dried in shade, ground using a laboratory grinder, and passed through a 20-mesh sieve (840 μm). Chicken manure was available from CCS HAU, Hisar.

Experimental setup

Clay pots of 1 kg capacity each were filled with autoclaved soil. Neem cake, neem leaves, and chicken manure were combined with *F. composticola* (Table 1). Organic amendments were mixed in pots for 15 days before sowing and light irrigation was applied. Three non-amended control treatments, viz., *M. incognita* alone, *F. composticola* alone, and un-inoculated were kept. Pots were arranged in a completely randomized design, 4 replicates per treatment. Two seeds of cucumber (cv. CCH-1) were sown in each pot and 1 week after germination, one plant/pot was retained. One-week-old seedlings were inoculated by pencil's hole method, at 2000 freshly hatched J_2 of *M. incognita* and 400 *F. composticola* per pot.

Maintenance of plants

After germination, plants were regularly watered and general care was taken. Hoagland solution was applied at 10 days' interval. Hand hoeing was carried out at desired intervals for proper aeration. Yellow trap cards were installed to manage the whitefly population and 3 sprays of Nimbicidin at 0.2% were applied to protect the crop from insects.

Observations

Observations were recorded 45 days after inoculation on plant height, shoot weight, root weight, number of galls, egg masses, and final nematode population in the soil. The number of galls and egg masses on roots were counted with the help of the magnifying lens. Compound galls were counted as single gall. Nematode populations were recorded at ×40 magnifications using a stereoscopic binocular microscope.

Temperature data

Weekly temperature data of the experimental period were obtained from the Department of Agricultural Meteorology, CCS HAU, Hisar, and presented in Fig. 1.

Statistical analysis

Data were subjected to analysis of variance (ANOVA), and the means were compared with a critical difference. A significance level of $P \leq 0.05$ was used in all analyses. Nematode data were transformed, using square root transformation to homogenize error variances. All calculations were performed with the OPSTAT software available online at the website of CCS HAU, Hisar (www.hau.ac.in).

Results and discussion

The present study shows that the population of *M. incognita* recovered after harvest was significantly lower (73.75/200 cc) in treatment where a combination of 3 amendments (chicken manure + neem cake + neem leaves) were incorporated into the soil than the treatments, where 2 amendments [neem cake + neem leaves (111.25/200 cc); chicken manure + neem leaves (131.25/200 cc); chicken manure + neem cake (168.75/200 cc)] were added to the soil. The use of organic amendments is effective and environmentally safe management of plant-parasitic nematodes and many other crop pests

Table 1 Effect of *Fictor composticola* and organic amendments in different combinations on growth parameters of cucumber infected with *Meloidogyne incognita*

Treatments	Plant height (cm)	Fresh shoot weight (g)	Fresh root weight (g)
F. composticola + neem cake 7.5 g/pot + neem leaves 7.5 g/pot + *M. incognita*	29.55	28.25	3.95
F. composticola + chicken manure7.5 g/pot + neem cake7.5 g/pot + *M. incognita*	32.28	28.59	3.80
F. composticola + neem leaves7.5 g/pot + chicken manure7.5 g/pot + *M. incognita*	30.35	28.19	3.68
F. composticola + chicken manure 5.0 g/pot + neem cake 5.0 g/pot + neem leaves 5.0 g/pot + *M. incognita*	49.18	42.73	5.74
F. composticola alone	25.73	15.07	2.41
Inoculated control (*M. incognita* alone)	16.08	4.65	3.11
Un-inoculated control	25.70	17.99	4.80
CD at 5%	3.12	2.58	0.90

F. composticola, 400 individuals per kg of soil
Root-knot nematode inoculum, 2000 J_2/kg soil

and pathogens (Atolani and Fabiyi 2020). Various organic amendments release nematoxic compounds such as organic acids, nitrogenous compounds like ammonia, or various plant secondary metabolites during their decomposition (Rodriguez-Kabana et al. 1987; Akhtar and Malik 2000).

Plant growth

Data of plant height, shoot weight, and root weight are presented in Table 1. Plant height in all treatments was significantly taller than the plants inoculated with the root-knot nematode alone. The maximum plant height (49.18 cm) was recorded in case of *F. composticola* + chicken manure + neem cake + neem leaves. The plant height was taller in the plants inoculated with *F.composticola* alone than those inoculated with *M. incognita* alone, although it was statistically at par with un-inoculated plants. Height was similar in the plants inoculated with chicken manure, neem cake, and neem leaves, in combinations of 2, but more than that inoculated with *M. incognita* or un-inoculated.

Fresh shoot weight was higher in all the treatments than in case of *M. incognita* alone. Shoot weight in *F. composticola* + chicken manure + neem cake + neem leaves was significantly higher than at all other treatments. All combinations of organic amendments in the presence of *F. composticola* were found significantly superior over un-inoculated check in improving plant weight. All the treatments having different combinations of amendments with *F. composticola* were statistically at par with each other, though they had more shoot weight than the un-inoculated check. Shoot weight in inoculated control was minimum (4.65 g) and significantly lower than all other treatments.

Root weight was maximum (5.74 g) in the treatment having *F. composticola* with chicken manure + neem cake + neem leaves, which was significantly higher than all other treatments. Fresh root weight in all the treatments was higher than *F. composticola* alone. Root

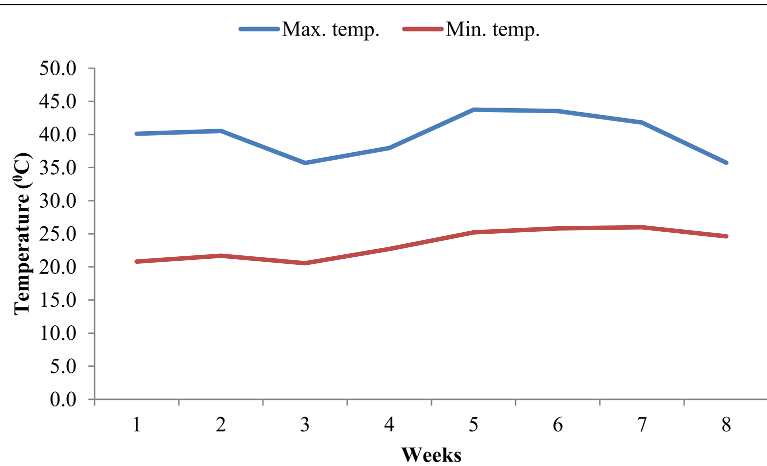

Fig. 1 Maximum and minimum temperature during the experimental period (3.5, 2019-16.6, 2019)

weight in the treatments, received combinations of the 2 organic amendments was similar but less than at un-inoculated control (Table 1).

When *F. composticola* was combined by organic amendments, the results showed that plant growth characteristics increased in all treatments than at the treatment inoculated with root-knot nematode alone. Plant growth in *F. composticola* + chicken manure + neem cake + neem leaves was significantly higher than all other treatments (Table 1). All treatments having different combinations of amendments with *F. composticola* had higher plant growth than un-inoculated check and it was minimum in inoculated control (Fig. 2).

Root-knot nematode

Numbers of galls, egg masses, and root-knot nematode populations are presented in Table 2. Indiscrete galls were observed on cucumber roots receiving various treatments where root-knot nematode was inoculated (Fig. 3). All the amended treatments showed significantly lesser gall formation than the inoculated control.

Minimum egg masses (11.75) were recorded in case of *F. composticola* + chicken manure + neem cake + neem leaves, while the maximum (157.5) was recorded in inoculated check. There was an overall decline in the mean egg masses counts in all treatments over an inoculated check and all the treatments differed significantly from inoculated check but they were at par with each other.

Soil population of the root-knot nematode was the lowest (73.75/200 cc) in case of *F. composticola* + chicken manure + neem cake + neem leaves and it was statistically lower than other treatments. The highest nematode count (1091.25 per 200 cc soil) was recorded in nematode inoculated control. In all other treatments, the nematode population was significantly lower than at the inoculated control.

Obtained results revealed that a minimum number of galls, number of egg masses, and nematode reproduction were recorded in the treatment of *F. composticola* + chicken manure + neem cake + neem leaves, while the maximum was found in inoculated check (Table 2). Nematode's population in all treatments was less than in the plants inoculated with *M. incognita* alone. Application of organic amendments along with *F. composticola* resulted in a fewer number of galls and egg masses than *M. incognita* alone. Chavarria-Carvajal et al. (2001) found that combinations of organic amendments and benzaldehyde were effective in reducing *M. incognita* and other plant-parasitic nematodes. However, numbers of root-knot nematodes were lower due to metabolites released through the decomposition of organic amendment and predatory nematode populations were able to have more impact and further reduce root-knot nematode numbers.

Population of predator

The predator's population was affected by the combinations of chicken manure, neem cake, and neem leaves as amendments. The population reached the maximum (38.75 per 200 cc soil) in the integrated treatment and the minimum (11.5/200 cc) in the case of *F. composticola* alone treatment (Fig. 4).

In the present study, a high population of the predatory nematode was recovered where chicken manure was added to the soil than other organic amendments. Perhaps it supported the higher microbial population,

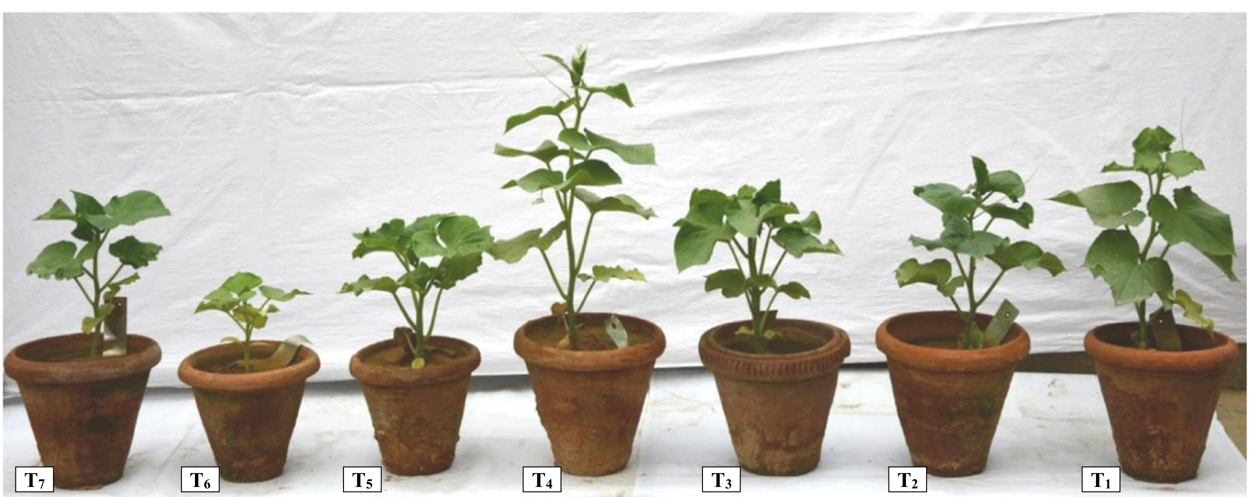

Fig. 2 Effect of *Fictor composticola* and organic amendments in different combinations on plant growth of cucumber infected with *Meloidogyne incognita*. T1, *F. composticola* + neem cake + neem leaves; T2, *F. composticola* + chicken manure + neem cake; T3, *F. composticola* + neem leaves + chicken manure; T4, *F. composticola* + chicken manure + neem cake + neem leaves; T5, *F. composticola* alone; T6, inoculated control; T7, un-inoculated control

Table 2 Effect of *Fictor composticola* and organic amendments in different combinations on gall formation and reproduction of *Meloidogyne incognita*

Treatments	Number of galls/plant	Number of egg masses/plant	Final nematode population/200 cc soil
F. composticola + neem cake7.5 g/pot + neem leaves 7.5 g/pot + *M. incognita*	17.75 (4.32)	11.75 (3.57)	111.25 (10.57)
F. composticola + chicken manure7.5 g/pot + neem cake 7.5 g/pot + *M. incognita*	37.50 (6.19)	21.00 (4.68)	168.75 (12.97)
F. composticola + neem leaves7.5 g/pot + chicken manure7.5 g/pot + *M. incognita*	29.00 (5.46)	18.25 (4.37)	131.25 (11.40)
F. composticola + chicken manure 5.0 g/pot + neem cake 5.0 g/pot + neem leaves 5.0 g/pot + *M. incognita*	17.25 (4.26)	10.00 (3.30)	73.75 (8.63)
Inoculated control (*M. incognita* alone)	82.00 (9.02)	157.50 (12.47)	1091.25 (32.99)
CD at 5%	(1.18)	(1.42)	(2.12)

Figure in the parentheses are square root transformed values

especially bacteria, which are fed by *F. composticola*. With the addition of organic amendments, increased populations of bacterivorous nematodes were obtained because of higher populations of bacteria during the process of decomposition (Bulluck and Ristaino 2002). Similar results were obtained by Wachira et al. (2009) who suggested that more populations of predacious nematodes were recorded in the soils amended with chicken manure in tomato crop. The carnivorous *Clarkus papillatus* was the most abundant in amended farms in comparison to conventional farms. With a cruciferous cover crop, the numbers of carnivorous *Mylonchulus sigmaturus* (*M. sigmaturus*) were the highest (Van Diepeningen et al. 2006).

In the present study, the final population of the predatory nematode was less than its initial population. This may be due to the high temperature during the experimental period, which was unfavorable for *F. composticola* (Keshari 2016). The present results also support the findings of Ahmad and Jairajpuri (1982) that the minimum population of predatory nematodes was found when encountered with the maximum soil temperature. There was a short generation time of *F. composticola* than *M. incognita* (Bajaj and Kanwar 2015). It may also be hypothesized that when *F. composticola* alone with *M. incognita* was added to the pots, the root-knot larvae escaped predation by entering roots because penetration by most root-knot larvae into roots can be accomplished within 48 h (Gourd et al. 1993). Thus, it seems clear that the non-availability of prey linked to reduce predatory nematode numbers at the end of the experiment. The final population density of *F. composticola* decreased than the initially added density. Obtained results are in harmony with those reported by Van Diepeningen et al. (2006) and Hu and Qi (2010) who found that omnivorous and predatory nematodes first increased after amending the soil and then decreased with time. In contrast, there was a twofold increase in *Mononchoides*

Fig. 3 Effect of *Fictor composticola* and organic amendments in different combinations on gall formation by *Meloidogyne incognita* on cucumber roots. T1, *F. composticola* + neem cake + neem leaves; T2, *F. composticola* + chicken manure + neem cake; T3, *F. composticola* + neem leaves + chicken manure; T4, *F. composticola* + chicken manure + neem cake + neem leaves; T5, *F. composticola* alone; T6, inoculated control; T7, un-inoculated control

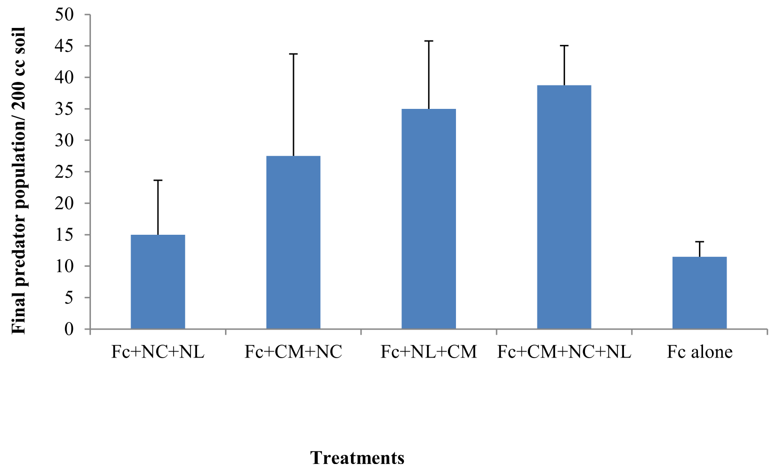

Fig. 4 Final population of *Fictor composticola* in presence of different combinations of organic amendments (NC, neem cake; CM, chicken manure; NL, neem leaves; Fc, *F. composticola*)

fortidens (*M. fortidens*) between inoculation and harvest during pot experiments (Khan and Kim 2005). Lal et al. (1983) also found the number of the predator population increased by about twofold markedly with the input of green manure to the soil.

Conclusions

All combinations of organic amendments in the presence of *F. composticola* were effective in suppressing root-knot nematode population as well as improving plant growth. The predator population was also maximum in the treatment where a combination of 3 amendments (chicken manure + neem cake + neem leaves) was incorporated into the soil. The results of this study seem to indicate that predatory nematode, *Fictor composticola*, and organic amendments could be considered potential candidates as alternative to pesticides, mainly suitable to organic farming, where a few and poorly effective tools are available not only for the control of soil pests and pathogens but also eligible for sustainable nematode management in conventional crops.

Abbreviations

F. composticola: *Fictor composticola*; *M. fortidens*: *Mononchoides fortidens*; *M. incognita*: *Meloidogyne incognita*

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Authors' contributions

The concept and design of the experiments were prepared by both authors. HSS conducted the experiments, analyzed the results, and wrote the manuscript. RSK supervised the results analysis and corrected the manuscript draft. The authors read and approved the final manuscript.

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Availability of data and materials

All data generated and/or analyzed during the present study are available in the manuscript, and the corresponding author has no objection to the availability of data and materials.

Ethics approval and consent to participate

The study was conducted on predatory and plant-parasitic nematode species that are abundant in the environment and does not require ethical approval.

Consent for publication

Not applicable

Competing interests

Both authors declare that they have no conflict of interest.

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