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RESEARCH ARTICLE

Effect of different fungal bio-agents on plant growth and yield attributes of tomato (*Lycopersicon esculentum* Mill.) against root-knot nematode, *Meloidogyne incognita* under field condition

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Abstract

The experimental trial was carried out in the Department of Nematology at the Rajasthan College of Agriculture, Udaipur, during the *Rabi* season in 2021–2022 and 2022–2023. The prime goal was to address the effects of different fungal bio-agents on tomato plant growth and yield attributes in challenged with the root-knot nematode (RKN). The trial followed to randomized block design (RBD) consisted seven treatments *viz.,Trichoderma harzianum, T. viride, Purpureocillim lilacinum*, *Metarhizium anisopliae* and *Baeuveria bassiana* as soil application (SA) at 5 kg/ha and seedling treatment (ST) at 5 g/L of water and carbofuran 3G 2 kg /ha as well as untreated check were also maintained with four replications. All the treatments significantly improved the plant growth parameter and yield as compared to untreated check. However, significant reduction in nematode population and maximum improvement in plant growth parameters was recorded with carbofuran followed by bio-agents. The results revealed that among different promising fungal bio-control agents tested, the highest biometric parameters like, shoot length (69.62 cm), fresh shoot weight (53.50 g), root length (37.87 cm), fresh root weight (27.87 g), yield (34.26 kg/3×4 plot). In addition, the promising module mitigating nematode reproduction like, no. of galls per plant (61.25), no. of egg masses per plant (55.50), no. of eggs & larvae per egg mass (174.12), nematode population per 200 cc soil (960.37), final nematode population (soil + plant) (10686.78), root gall index (1-5 scale). The reproductive factor (R_f) (22.32) was recorded in *T. harzianum* as SA at 5 kg/ha and ST at 5 g/lit of water (pooled data of 2021-22 and 2022-23, respectively) followed by *T. viride* and *P. lilacinum* in both years.

Keywords: bio-management; biometric parameters; root gall forming parasite; Solanaceae crop

Introduction

Vegetable crops are globally cultivated as a rich source of nutrients and fiber to enhance the human diet (1). Tomato (*Lycopersicon esculentum* Mill.) is one the significant vegetable crop grown all over the world. The tomato is also referred to as "a poor man's orange". In India, tomato is cultivated on about 25.66 million hectares with a production of 320.48 million tonnes and productivity of about 25.2 tonnes per hectare (2). Andhra Pradesh, Madhya Pradesh, Karnataka, Gujarat, Odisha, West Bengal, Chhattisgarh, Bihar, Telangana, Tamil Nadu, Uttar Pradesh, Maharashtra, Haryana and Himachal Pradesh are the main tomato-producing states in India. In India, notably 90 % of tomato production comes from these states. In Rajasthan, the area under tomato cultivation was estimated at 12.62 thousand hectares with production of 45.51 thousand metric tonnes and productivity about 13.83 t/ha (3). Major tomato growing districts in Rajasthan include Ajmer, Alwar, Jaipur, Jalore, Sirohi, Sikar and Udaipur. Tomato is a superfood loaded with rich sources of minerals, organic acids and vitamins A, B and C.

The crop is prone to numerous insect and non-insect pests. In addition to insect pest and diseases, plant parasitic nematodes have also become a limiting factor in the successful cultivation of tomatoes. Among several plant parasitic nematodes recorded, root-knot nematode (RKN), *Meloidogyne incognita* is a highly devastating and notorious pest in horticulture crops poses severe yield losses throughout the world (4-5). Considering they can infiltrate multiple crop species, root-knot nematodes have caused significant yield losses of several abundant crops internationally (6-7). Overall, PPNs cause 21.3 % crop losses amounting to ₹102039.79 million ($1.58 billion) annually (8). RKN, *M. incognita* causing 23 % yield losses of ₹6035.2 million in tomatoes. In addition, it usually promotes typically root galling, leaf yellowing, defoliation, stunting and wilting of infected plants (9).

To manage the RKN, different types of tactics have been investigated against the root-knot nematode (10). However, these tactics have not been effective against nematodes. Nematicide is a practical strategy for RKN control (11-12). Although indiscriminate use of nematicides led to negative impacts on human health and the environment. The use of bio -control agents is one viable and supporting alternative to nematicides (13). Predatory nematodes, antagonistic fungi and egg-parasitic fungi are some of the biological control agents that have been studied. These bio-agents are effective in inhibiting the growth of plant diseases and promoting the growth of agricultural products (14-17).

Therefore, the current research goal is to evaluate the potential antagonistic effects of commercial formulations of bio-agents of *Trichoderma harzianum, T. viride, Purpureocillim lilacinum*, *Metarhizium anisopliae* and *Baeuveria bassiana* against *Meloidogyne incognita* their impact on tomato growth and development in field conditions and also it has mitigation activity against nematodes.

Materials and Methods

Experimentation area and atmospheric conditions

The experimental trial was carried out in the Department of Nematology at the Rajasthan College of Agriculture, Udaipur, during the *Rabi* season in 2021-2022 and 2022-2023. Plot dimensions were 3 x 2 m, with a 60 x 60 cm spacing. This region receives 76 to 90 cm of rain on average year. Rainfall occurs primarily from mid-June to mid-September, with sparse winter showers.

Source of fungal bio-agents

Trichoderma harzianum, T. viride and *Baeuveria bassiana* were procured from MPUAT, RCA, Udaipur and *Purpureocillim lilacinum*, *Metarhizium anisopliae* were procured from TNAU, Tamil Nadu were used in this study.

Soil sampling

To determine the initial population of root-knot nematodes (450 J_2/200 cc soil and 510 J_2/200 cc soil - 2021-22 and 2022-23, respectively), a random soil sample was taken at a depth of approximately 15-20 cm from the plant root zone using khurpi from 4-5 locations in the naturally infested field. The soil was then homogenized, filled in a polythene bag, labeled, tied and brought to the laboratory for additional processing.

Nematode's extraction

Nematode from soil was extracted by Cobb's method (18). This was followed by the modified Baermann funnel method (19). The funnel technique uses a supporting to hold the plant tissue partly submerged in water to avoid anaerobic decomposition. A paper tissue is placed on the support and the chopped plant material is placed on it. Fill the funnel with tap water and set the sieve in the funnel to partly submerge the filter in the water. Samples must not be completely submerged in water. Drain off sufficient water if necessary. After 24-48 hr, collect the nematode suspension for counting.

Staining of roots and preparation of perineal pattern

The roots were completely cleaned under running water to get rid of any remaining soil particles and they were subsequently boiled for two to three minutes in 0.1 % acid fuchsin lactophenol dye. Roots were gently washed in tap water to remove excess dye and then they were placed in lactophenol for an entire night to destain (20). The roots were examined using a stereoscopic binocular microscope and *M. incognita* was identified by teasing out the dyed females from the roots in order to prepare the perineal pattern (21).

Treatment details

The experiment was conducted to examine the effect of bio-agents on root-knot nematode, *M. incognita* on tomato under naturally infested field conditions. The experiment was laid out in a randomized block design (RBD) with seven treatments *i.e.,* T1- *T. harzianum* as soil application (SA) at 5 kg/ha. and seedling treatment (ST) at 5 g/L of water, T2- *T. viride* as soil application (SA) at 5 kg/ha. and seedling treatment (ST) at 5 g/L of water, T3- *P. lilacinus* as soil application (SA) at 5 kg/ha. and seedling treatment (ST) at 5 g/L of water, T4- *M. anisopliae* as soil application (SA) at 5 kg/ha. and seedling treatment (ST) at 5 g/L of water, T5- *B. bassiana* as soil application (SA) at 5kg/ha and seedling treatment (ST) at 5g/L of water, T6- Carbofuran 3G 2 kg a.i /ha as were applied as check and T7- Untreated control was also maintained for the comparison with four replications. Observations such as biometric parameters *viz.,* fresh shoot and root length (cm), fresh shoot & root weight (g), yield (kg/plot) and nematode multiplication *i.e.,* no. of galls/plant, no. of egg masses/plant, no. of eggs/larvae per egg-mass, nematode population/200 cc soil, final nematode population (soil + plant), root gall index (RGI) and reproduction factor value (R_f) at harvesting.

Results

Effect of fungal bio-agents on plant growth parameters and yield attributes of tomato

The result of experiment revealed that among the different fungal bio-agents, T_1- *T. harzianum* as SA at 5 kg/ha and ST at 5 g/L of water challenged with root-knot nematode in tomato crop at the time of harvesting was more effective in promoting the plant growth of tomato like, shoot length (70.00 cm and 69.25 cm), root length (38.75 cm and 37.00 cm), fresh shoot weight (54.25 g and 52.75 g), fresh root weight (28.75 g and 27.00 g) and yield (35.37 and 34.25 kg/plot) in 2021-22 and 2022 -23 respectively. Similarly, the tomato plants treated with T_2- *T. viride*as SA at 5 kg/ha and ST at 5 g/L of water were found second best treatment in promoting the plant growth of tomato like, shoot length (58.75 cm and 57.25 cm), root length (24.50 cm and 22.25 cm), fresh shoot weight (43.25 g and 42.00 g), fresh root weight (20.50 g and 19.00 g) and yield (32.18 and 31.25 kg/plot) in 2021-22 and 2022-23 respectively and T_3- *P. lilacinum*as SA at 5 kg/ha and ST at 5 g/L of water were found third best treatment in increasing the shoot length (43.00 cm and 42.00 cm), root length (20.50 cm and 19.00 cm), fresh shoot weight (34.00 g and 32.00 g), fresh root weight (18.00 g and 16.50 g) and yield (25.31 and 24.00 kg/plot) in 2021-22 and 2022 -23 respectively. Whereas application of carbofuran 3G at 2kg a.i/ha kept as check noticed highest shoot length (78.00 cm and 76.75 cm), root length (44.25 cm and 42.75 cm), fresh shoot weight (65.75 g and 63.50 g), fresh root weight (33.50 g and 31.25 g) and yield (36.87 and 35.50 kg/plot) in 2021-22 and

2022-23 respectively (Supplementary Table 1).

Supplementary Table 2 and Fig. 1 show that percent changes in plant growth and nematode reproduction parameters over the untreated check. The highest percent increase in shoot length (207.06 %), root length (341.79 %), fresh shoot weight (307.43 %), fresh root weight (391.44 %) and yield (176.10 %) in carbofuran followed by bio-agents (pooled data of 2021-22 and 2022-23).

Effect of fungal bio-agents on nematode reproduction in plant and soil

The study revealed that the minimum number of nematode reproduction like, number of galls per plant (60.25 and 62.25), number of egg masses per plant (54.75 and 56.25), number of eggs & larvae per egg mass (172.50 and 175.75), nematode population per 200 cc soil (958.25 and 962.50), final nematode population (soil+plant) (10462.87 and 10910.68), root gall index (1-5 scale) (3 and 3) and reproductive factor (23.25 and 21.39) were observed in T_1- *T. harzianum* as SA at 5 kg/ha and ST at 5 g/L of water in 2021-22 and 2022-23, respectively. Similarly, the tomato plants treated with T_2- *T. viride*as SA at 5 kg/ha and ST at 5 g/L of water were revealed second best treatment in suppressing of number of galls per plant (72.75 and 74.50), number of egg masses per plant (60.75 and 63.50), number of eggs & larvae per egg mass (193.25 and 195.25), nematode population per 200 cc soil (1016.30 and 1019.25), final nematode population (soil+plant) (12828.98 and 13492.12), root gall index (1-5 scale) (3 and 3) and reproductive factor (28.50 and 26.45) in 2021-22 and 2022-23, respectively and T_3- *P. lilacinum*as SA at 5 kg/ha and ST at 5 g/L of water were found third best treatment in reducing the number of galls per plant (80.25 and 84.25), number of egg masses per plant (72.50 and 74.25), number of eggs & larvae per egg mass (202.25 and 206.25), nematode population per 200 cc soil (1042.50 and 1049.25), final nematode population (soil+plant) (15735.31 and 16447.56), root gall index (1-5 scale) (4 and 4) and reproductive factor (34.96 and 32.25) in 2021-22 and 2022-23, respectively. Whereas application of carbofuran 3G at 2 kg a.i/ha kept as check noticed lowest number of galls per plant (46.75 and 48.50), number of egg masses per plant (35.25 and 37.25), number of eggs & larvae per egg mass (154.50 and 155.25), nematode population per 200 cc soil (848.25 and 852.25), final nematode population (soil+plant) (6341.12 and 6683.81), root gall index (1-5 scale) (2 and 2) and reproductive factor (14.09 and 13.10) in 2021-22 and 2022-23, respectively (Supplementary Table 3).

The highest per cent decrease over control in nematode reproduction like, number of galls per plant (60.92 %), number of egg masses per plant (67.53 %), number of eggs & larvae per egg mass (37.79 %), nematode population per 200 cc soil (37.37 %), final nematode population (soil+plant) (77.96 %) followed by bio-agents (pooled data of 2021-22 and 2022-23) (Supplementary Table 2, Fig. 2).

Discussion

The current study results revealed that all fungal bio-agents were significantly enhancing the biometric parameters as well as yield and reducing the nematode population. Among the different fungal bio-agents, T_1- *T. harzianum* as SA at 5 kg/ha and ST at 5g/L of water were recorded highest plant growth parameters *viz.,* shoot length (cm), shoot weight (g), root length (cm), root weight (g) and yield (kg/plot) followed by T_2 - *T. viride* and T_3 -*P. lilacinum*in mitigating of root-knot nematode and production of high yield of tomato. Results are agreement with the previous studies found that significantly higher plant growth was recorded in *Trichoderma harzianum, T. flavus* and *P. lilacinum* applied as seed/soil drench compared to control and lowest in *P. fluorescence* treated plants (22). *Trichoderma* spp. is utilized in the production of a number of antibiotics, such as trichoderin, trichodermol A and harzianolide. *Trichoderma* produces molecules such as 6-pentyl α-pyrone, VOCs and enzymes that can attack the cuticle of nematodes (23). Successful parasitism of the nematode by *Trichoderma* requires mechanisms to facilitate penetration of the nematode cuticles or eggshells. The involvement of lytic enzymes has long been suggested and demonstrated in *Meloidogyne* parasitism

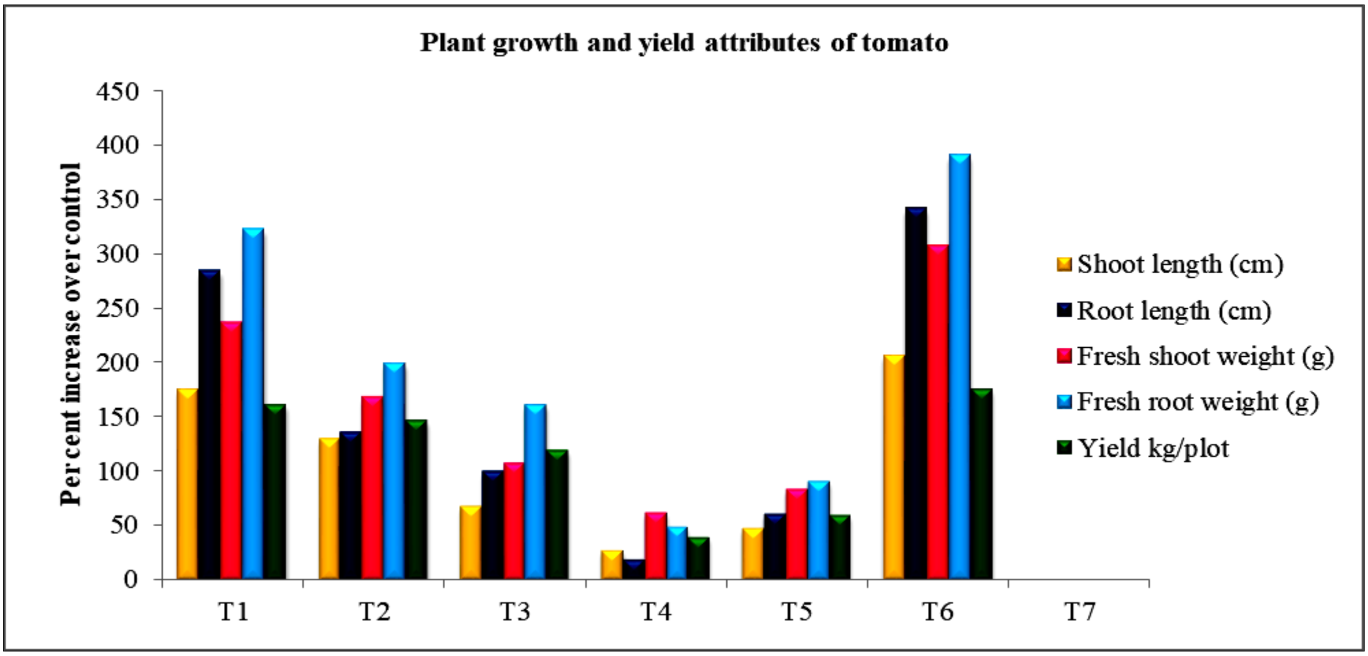

Fig. 1. Percent changes in plant growth and yield attributes through application of fungal bio-agents against root-knot nematode, *Meloidogyne incognita* infecting tomato under field condition (pooled data of 2021-22 and 2022-23).

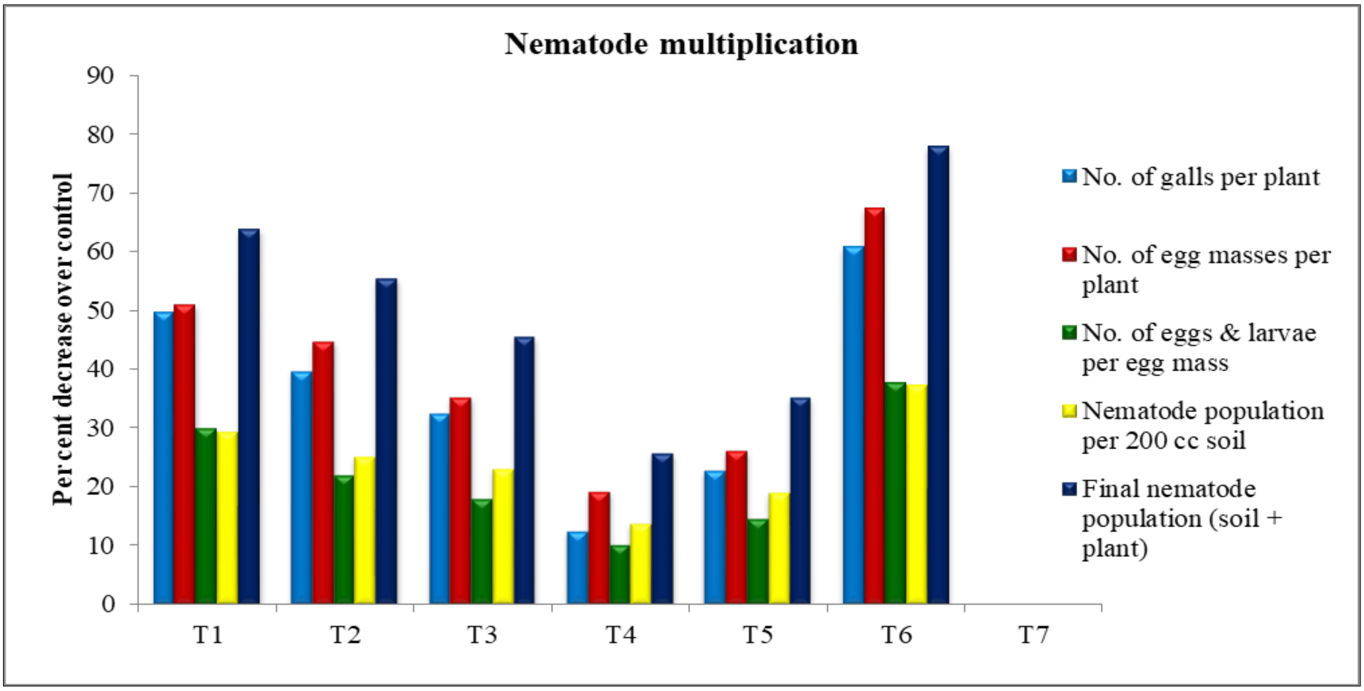

Fig. 2. Percent changes in root-knot nematode, *Meloidogyne incognita* reproduction through application of fungal bio-agents in tomato under field condition (pooled data of 2021-22 and 2022-23).

(24). *T. harzianum* is an effective bio-control agent for the management of root-knot and other nematodes (25-28). These findings were also agreement with the earlier studies that neem cake and *T. harzianum* significantly increased plant growth and reduced root galling and final population of root-knot nematode in tomato seedlings transplanted in neem cake amended soil incorporated with *T. harzianum* (29). Soil treated with *T. harzianum* reduced the disease by 92 % (30). Similarly, previous research revealed that *T. harzianum* when applied to tomato increased the height and dry root weight (31).

The root-knot index and the number of egg masses were significantly reduced upon three applications of *T. viride* (32). In concurrence with the current study, *T. viride* and *P. lilacinus* were applied in combination at half the dose (1 g/500 g soil) reduced nematode population (80 L/500 gm soil) as compared to *P. lilacinus* (190 L/500 g soil) or *T. viride* (230 L/500 g soil) alone and plant growth significantly increased in *T. viride* alone and in combination of *T. viride* + *P. lilacinus* (24.5 cm and 25.1 cm respectively) as compared to *P. lilacinus* alone (21.5 cm) (33). The soil application of *T. viride* was able to control the nematode population and improve the mulberry leaf yield and nutritional standards (34). The indigenous isolates of *T. harzianum* and *T. viride* have the potential to control *M. incognita* (35). Earlier data reported that *T. viride* or *T. harzianum* when mixed in soil at 1 g/kg soil improved plant height, shoot weight, root length, root weight and reduced *M. incognita* population in tomatoes (36).

After treatment of *Trichoderma* spp. next best treatment is T_3- SA (5kg/ha.) + ST (5g/lit of water) with *P. lilacinus* also decreases root-knot and increases plant growth parameters due to the production of 'paccilotoxin' and 'lilacin' antibiotic and egg parasitic nature of *M. incognita*. In concurrence with the current study, the datarevealed that *P. lilacinum* at 1.0 g/kg had a promising impact against *M. incognita* and a significant reduction of nematode population in soil, number of developmental stages, females and number of egg masses on Cucumber plants comparing with the other treatments (37). *P. lilacinum* is mainly an egg parasite. The fungus produces antibiotics *viz*., leucinostatin and lilacin and enzymes such as protease and chitinase. Protease has nematicidal activity, causes degradation of the eggshell and inhibits hatching. Chitinase breaks down the eggshell making the route for the fungus to pass through. The developing juvenile inside the egg is destroyed by the rapidly growing hyphae. Many conidiophores are produced and the hypha moves to the adjacent eggs (38).

The potential of *B. bassiana*, on the one hand, it reduces the nematode population, on the other hand, it can amend the plant growth parameters due to the secretion of a toxic chemical such as 'beauverin' and may be also its egg parasitic nature. This is supported by the previous findings (39). *B. bassiana* has a repressive action on nematodes of the genus *Meloidogyne* spp. (40-46). *B. bassiana* may have more than a single bioactive metabolite that are responsible for nematicidal activities and each metabolite may act on a different site. Other findings purified the isolated fungus and also they showed the bio-control potential of the isolate on *Heterodera avenae*, with 47.1 % of larval mortality (47). Studies have shown that *Beauveria* can produce beauvericin and oosporin. Beauvericin has a weak activity against *M. incognita* (48-51). The percentage mortality and inhibition of hatching of root-knot nematode were directly proportional to the concentration of culture filtrates of *B. bassiana* (52).

The bio-control potential of *M. anisopliae* against some species of root knot nematodes has been shown (53-55). The lethal effect of *M. anisopliae* culture extract has been also reported (56). Some species of *Metarhizium* have root colonization ability (57-58). Some isolates of *M. anisopliae* have endophytic behaviour (59). The fungus produces sticky conidia that attach to the nematode cuticle (60). The conidia germinate, parasitize and kill the cadaver, by direct penetration and producing the infective hyphae inside the nematode body. The fungus produces cyclopeptides and destruxins which play a crucial role in its pathogenicity (61).

Before any direct attack on the host, the fungus produces destruxin A and destruxin B that can kill the host (62).

Conclusion

Keeping in view the negative impacts of chemical nematicides on the environment, bioagents have come up as an eco-friendly and sustainable approach of controlling nematodes in important crops like tomatoes. This research work has focussed on the nematicidal effect of different fungal bio-agents on plant growth and yield attributes of tomato against root-knot nematode, *Meloidogyne incognita* under field conditions. The result of the current study revealed that various fungal bio-agents applied as soil application at 5 kg/ha and seedling treatment at 5 g/L of water can be suitable candidates for use in the biological control of *M. incognita*. It is very imperative to lessen the consumption of pesticides and enhance sustainable agriculture notably fungal bio-agents could also have a potential impact on organic farming cultivation.

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Authors' contributions

RA carried out formal analysis, data curation and practical experimental work and drafted the manuscript. MKS carried out research conceptualization, methodology, supervision, design of the experiment. MS and BLB participated in the sequence alignment. DK and M carried out interpretation of the data, cross checking and editing. CPN and RNK participated in the proof reading and manuscript preparation.

Compliance with ethical standards

Conflict of interest: The authors have said that there were no conflicting agendas.

Ethical issues: None

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