Egyptian Journal of Biological Pest Control

RESEARCH

Open Access

Root-knot nematode pathogen suppression in eggplant using antagonistic fungi

Jalal Uddin[1], Farman Ullah[1], Ishrat Naz[2], Sarir Ahmad[1*], Ahmad-Ur-Rahman Saljoqi[1], Shahid Sattar Khan[1] and Muhammad Salim[1]

Abstract

Background *Meloidogyne incognita* (Kofoid and White) Chitwood is a major pest of eggplant in Pakistan. The second-stage juveniles (J2s) feed on the roots of eggplant. Damaged roots swell and the plant exhibits stunted growth. Mostly farmers rely on the use of broad-spectrum nematicides. However, due to hazardous effects on the environment and non-target species, its application is greatly criticized. Fungal biocontrol agents have been long used for curtailing root-knot nematode infections. The present study was conducted to determine the virulence of four fungal biocontrol agents against *M. incognita* under laboratory and greenhouse conditions.

Results The *in vitro* results revealed that *Trichoderma harzianum* significantly caused 77.60% egg inhibition, followed by *Pochonia chlamydosporium* (53.0%) at 1:1 concentration after 72 h. The maximum J2s mortality was achieved by *T. harzianum* (82.0%), followed by *P. chlamydosporium* (70.20%) at 1:1 concentration after 72 h. The *in-planta* examination carried out at a greenhouse demonstrated that the soil drench treatment of fungal biocontrol agents significantly suppressed *M. incognita* parameters and upraised the eggplant growth. The mean least number of galls (22.25) was in *T. harzianum* treated plants, while the control had (206.8) galls. Likewise, *T. harzianum* curtailed the least egg masses to 35.75 and swollen females to 21.12 than control negative (224.13 egg masses and 182.75 swollen females).

Conclusion *T. harzianum* was the best agent to control *M. incognita* (J2s) effectively, followed by *P. chlamydosporum*. *T. harzianum* may be a contribution to the biological control of *M. incognita* in Pakistan.

Keywords Biological control, *Meloidogyne incognita*, *Trichoderma harzianum*, *Aspergillus niger*, *Pochonia chlamydosproium*, *Penicillum chrysogenum*, Eggplant

Background

Eggplant (*Solanum melongena* L.) is a tropical and subtropical vegetable from the solanaceous (Rajan et al. 2022). It is widely cultivated, but key producers are in South East Asia including Pakistan, India, Japan, and China (Alam and Salimullah 2021). *S. melongena* faces enmity from both biotic and abiotic stresses including environmental hazards, pests including pathogens like (fungi, viruses, bacteria, root-knot nematode, and insects, *i.e.,* jassids, mites, thrips, fruit and shoot borer, mealybug, aphids, and whitefly (Elkelany et al. 2020).

Root-knot nematodes (RKN) are considered among the top five phytopathogens. RKN's genera are the tenth most prevalent genera of phyto-parasitic nematodes globally (El Aimani et al. 2022). *Meloidogyne* species are obligate sedentary endoparasitic RKNs that attack the underground plant tissues including the root system. They are polyphagous pests and are supposed to be cosmopolitan in nature. They also interact with several other phytopathogens that make them even more lethal and thus are responsible for hindering the high yield of *S. melongena* (Giri et al. 2022).

*Correspondence:
Sarir Ahmad
sarirplants@gmail.com
[1] Plant Protection Department, The University of Agriculture Peshawar, P.O. Box 25120, Peshawar, Pakistan
[2] Plant Pathology Department, The University of Agriculture Peshawar, P.O. Box 25120, Peshawar, Pakistan

The J2 stage is highly active and feeds within the cortex. RKN infection initiates with a root incursion by the J2 that is hatched from the eggs in the soil. The J2 punctures the epidermis of the cell wall with the help of stylet. It constantly moves around in the root intercellular spaces in search of establishing a suitable feeding site. Also, it remains in the vascular tube and the feeding site is established in giant cells due to the defense system activation from the host plant (Niu et al. 2022).

The RKN management plans are staggeringly more intricate than other phytopathogens because habitually parasitic nematodes conceal underground inhabitation in soil and root tissues, and thus escape the grower eye visibility. The chemical control is not feasible because of its low penetration into the RKN ovum, fast leaching down into the water table, and degradation issues. The high price of commercial nematicides makes it economically unfeasible to be utilized by the growers for the management of RKN. Over the past few decades, biological control gained interest to tackle the pest problem. The concept of biological control is considered a reliable substitute for toxic pesticides (Saad et al. 2022). Scientists have explored several antagonistic microorganisms that could be utilized as bio-control agents against the RKNs. Nematophagous fungi had a core action against the RKNs as they directly influence them by minimizing their population. Furthermore, these fungi may have a key role in the host plant growth promotion and could induce systemic resistance in plants (Yu et al. 2022). The use of biocontrol is highly effective because it could be simultaneously used with other control practices in the Integrated Pest Management (IPM). Keeping in view the significance of biocontrol agents for the management of RKNs, present in vitro and *planta* experiments were conducted to investigate the antagonistic properties of fungi, viz. *Trichoderma harzianum, Aspergillus niger, Penicillium chrysogenum, Pochonia chlamydosporium* against *M. incognita* in the laboratory and greenhouse on eggplant.

Methods

Six treatments including four fungi (*Trichoderma harzianum, Aspergillus niger, Pochonia chlamydosproium,* and *Penicillum chrysogenum* @ 2.5 g/1000 ml of water) were carried out. Positive check included the nematicide, Carbofuran (@ 1 g per plant). A negative check received simple distilled water (SDW) with nematode eggs and juveniles. Figure 1 shows microscopic photographs of the applied fungal treatments.

Host plant, fungi, and root-knot nematode (RKN)

Eggplant *cv. Local-Peshawar* was utilized as the host plant against the RKN. Seeds of eggplant were surface sterilized in Sodium hypochlorite (NaOCl) 1 percent solution for about 20 min and gently washed with SDW. The fungal suspensions of the selected biocontrol agents were provided by the Institute of Biotechnology and Genetic Engineering (IBGE), The University of Agriculture Peshawar Pakistan. A fungal culture was maintained on the freshly prepared Potato Dextrose Agar (PDA) medium under sterile conditions. The suspensions of fungi were identified based on conidium, color, colony texture, and shape (Darwesh et al. 2019). The Richards Liquid Medium (Fuchs et al. 2002) was used for the mass production of fungal bio-control agents. *M. incognita* was used as a pathogen of the eggplant host.

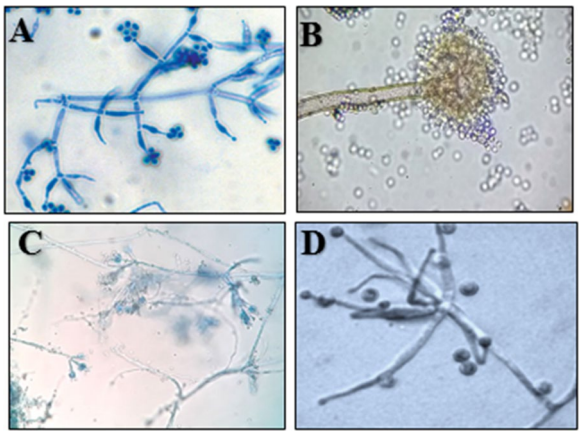

Fig. 1 Microscopic photographs of **A** *Trichoderma harzianum* **B** *Aspergillus niger* **C** *Penicillium chrysogenum* **D** *Pochonia chlamydosporium*

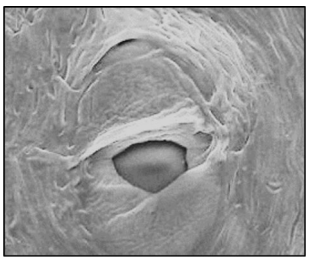

Fig. 2 A Scanning electron microscope (SEM) photographs of the perennial pattern present on the swollen female of *M. incognita*

Pure culture maintenance of *Meloidogyne incognita*

Pure culture of *M. incognita* was maintained on eggplant cultivar (Local-Peshawar) through single egg mass inoculation in the green house (Fatima et al. 2022). The swollen female body patterns (perennial patterns) were used for the identification (Fig. 2) (Sasser et al. 1983). Infected galls of eggplant collected from Malakand Division were isolated from the roots dipping the roots in 0.05 percent

NaOCl for 5 min (Barker and Hussey 1976). Then, eggs were collected and washed with SDW on 25-µm sieves. The egg masses were allowed to hatch in an incubator for the collection of J2s about 5 days later for their use in *in vitro* and *in-planta* studies.

In-vitro bioassay

The in vitro investigation was evaluated against the RKN egg masses and J2s in Petri plates. The Completely Randomized (CR) design was adopted and total of five replicates of each treatment was taken. The egg hatch inhibition and J2s mortality were recorded at the incubation periods of 24, 48, and 72 h by using the following formulae (Sikandar et al. 2020).

$$\text{Percentage of egg hatch inhibition} = \left(\text{No of eggs hatched}\right)/\left(\text{initial eggs}\right) \times 100.$$

$$\text{Percentage of J2 mortality} = (\text{No of dead J2s/total J2s}) \times 100\%.$$

Serial dilutions of each bio-control agent (1:1, 1:10, 1:100 V/V) were prepared in SDW. A total of 2 ml of SDW containing 6 egg masses and 200 freshly hatched J2s decanted into Petri plates having 8 ml of each dilution of the tested bio-control agents. SDW was used as negative control, while positive control received the nematicide and carbofuran. The Petri plates were covered with lids and enclosed in parafilm. Petri plates were kept at 28 °C (Khan et al. 2022a).

Greenhouse experiment

Seedling raising and transplantation

The eggplant seeds were sown in sterilized soil beds (sand: clay; 1:2). The two-week-old eggplant seedlings were transplanted to 50-cm diameter earthen pots (Dennis 2022).

Application of fungal biocontrol agents in a greenhouse experiment

The *in-planta* studies were laid-out in the glasshouse of Plant Pathology Section, Agricultural Research Institute (ARI) Turnab Farm Peshawar. The experiment was set up in a Completely Randomized (CR) design, having eight replications. The glasshouse condition was maintained at 25 °C, and RH was about 95–100%.

Approximately, 2200 J2s were inoculated to the earthen pots containing eggplant seedlings. The fungal suspension (1000 ml; containing 2.5 g mycelium + spores) was applied with the help of a sterile micro-pipette as a soil drench method (Naz et al. 2013). The eggplant plants inoculated with J2s were raised for two months and then uprooted for recording the data regarding RKN and plant growth parameters before experiment termination. The plants were uprooted and gently washed with SDW for removing debris of the soil particles. The Taylor and Sasser (1978) galling index (GI) was followed which was given below. The plants having no galls were indexed as 0, while the plant having over 100 galls was indexed as 5 (Oyetunde et al. 2022). The data on the eggplant growth parameters *i.e.,* root and shoot lengths/ weights, and no. of flowers, and RKN parameters *i.e.* egg masses and females were recorded.

Statistical analysis

Data regarding the egg hatch inhibition and J2 mortality were corrected with Abbot Formula (Chen et al. 2023); then, percent inhibition and mortality were calculated with the help of mentioned formulae. A statistical software STATISTIX (8.0 EL USA) was used for the analysis of the data. The least significant difference (LSD) test at $p = 0.05$ was used to find the F-ratio statistical variation among the fungal biocontrol agents..

Results

In vitro bioassays

In vitro bioassay for egg hatch inhibition and J2s mortality showed that the effectiveness of all the treatments increased with the passage of time as well as concentration. All the treatments were found statistically significant at $p = 0.05\%$ over a check with eggs and juveniles.

The means comparison of different fungal biocontrol agents at 24 h revealed that *T. harzianum* inhibited 37.6% of RKN eggs, followed by the standard carbofuran (32.4%) and then by *P. chlamydosporium*, which

Table 1 Percentage of egg hatching inhibition by various fungal biocontrol agents at varying concentrations at 24 h

Treatments	1:1	1:10	1:100	Mean (%)
Trichoderma harzianum	51.52%	34.95%	24.85%	37.66% a
Pochonia chlamydosporium	35.35%	25.66%	18.18%	27.13% c
Aspergillus niger	28.28%	19.19%	12.12%	20.60% d
Penicillum chrysogenum	19.19%	12.32%	7.68%	13.93% e
Carbofuran	46.46%	28.69%	20.20%	32.46% b
Control	1.20%	1.40%	0.80%	1.13% f
Mean (%)	30.80% a	21.00% b	14.66% c	

Each value is the mean of 5 replications. A letter followed by the same alphabet doesn't differ significantly

Table 2 Percentages of egg hatching inhibition by various fungal biocontrol agents at varying concentrations at 48 h

Treatments	1:1	1:10	1:100	Mean (%)
Trichoderma harzianum	63.19%	51.91%	40.74%	54.80% a
Pochonia chlamydosporium	47.66%	38.51%	27.66%	41.66% c
Aspergillus niger	36.60%	24.47%	18.09%	30.80% d
Penicillum chrysogenum	25.32%	19.15%	10.64%	23.26% e
Carbofuran (positive control)	59.79%	46.17%	35.74%	50.40% b
Control (negative control)	6.00%	6.00%	5.80%	5.93% f
Mean (%)	42.43% a	34.23% b	26.76% c	

Each value is the mean of 5 Replications. A letter followed by the same alphabet doesn't differ significantly

Table 3 Percentages of egg hatching inhibition by various fungal biocontrol agents at varying concentrations at 72 h

Treatments	1:1	1:10	1:100	Mean (%)
Trichoderma harzianum	75.65%	58.90%	47.83%	64.06% a
Pochonia chlamydosporium	61.74%	47.25%	37.17%	53.00% c
Aspergillus niger	50.87%	36.09%	25.22%	42.60% d
Penicillum chrysogenum	40.00%	30.33%	19.13%	35.66% e
Carbofuran (positive control)	66.96%	54.29%	44.13%	58.86% b
Control (negative control)	7.80%	8.60%	8.00%	8.13% f
Mean (%)	53.23% a	43.33% b	34.60% c	

Each value is the mean of 5 Replications. A letter followed by the same alphabet doesn't differ significantly

Table 4 Percentages of J2 mortality by various fungal biocontrol agents at varying concentrations at 24 h

Treatments	1:1	1:10	1:100	Mean (%)
Trichoderma harzianum	64.49%	55.92%	45.71%	56.26% a
Pochonia chlamydosporium	56.94%	46.94%	36.53%	47.86% c
Aspergillus niger	43.67%	35.92%	26.73%	36.73% d
Penicillum chrysogenum	29.39%	20.61%	15.10%	23.26% e
Carbofuran(positive control)	61.43%	50.61%	42.04%	52.33% b
Control(negative control)	1.80%	2.20%	1.60%	1.86% f
Mean (%)	43.76% a	36.33% b	29.06% c	

Each value is the mean of 5 replications. A letter followed by the same alphabet doesn't differ significantly

Table 5 Percentages of J2 mortality by various fungal biocontrol agents at varying concentrations at 48 h

Treatments	1:1	1:10	1:100	Mean (%)
Trichoderma harzianum	73.67%	63.88%	56.73%	65.46% a
Pochonia chlamydosporium	63.27%	55.10%	45.92%	55.66% c
Aspergillus niger	52.24%	42.86%	34.90%	44.46% d
Penicillum chrysogenum	40.61%	30.20%	22.65%	32.53% e
Carbofuran(positive control)	69.59%	61.43%	52.04%	61.80% b
Control(negative control)	2.40%	2.40%	2.20%	2.33% f
Mean (%)	50.96% a	43.46% b	36.70% c	

Each value is the mean of 5 replications. A letter followed by the same alphabet doesn't differ significantly

inhibited 27.1% of eggs. *A. niger* (20.6%) was the least number of eggs inhibited (Table 1).

The means comparison of different fungal biocontrol agents at 48 h revealed that *T. harzianum* inhibited 57.8% of RKN eggs, followed by the standard carbofuran (50.4%) and then by *P. chlamydosporium*, which inhibited 41.6% of eggs. The least number of eggs was inhibited by *A. niger* (30.8%), followed by *P. chrysogenum* (23.2%) (Table 2).

The means comparison of the results of different fungal biocontrol agents at 72 h indicated that *T. harzianum* inhibited 64% RKN eggs, followed by the standard carbofuran (58.8%) and then by *P. chlamydosporium* that inhibited 53.0% eggs. The followed treatments were *A. niger* and *P. chrysogenum* which inhibit 42.6 and 35.6% RKN eggs, respectively, at a $p=0.05\%$ significance level (Table 3).

The means comparison of percentage of J2s mortality results of different fungal biocontrol agents at 24 h of incubation indicated that *T. harzianum* caused maximum mortality (56.2%), followed by the standard carbofuran (52.3%) and then by *P. chlamydosporium* that caused (47.8%) J2s mortality. The, following treatments were *A. niger* and *P. chrysogenum* causing 36.7 and 23.2% RKN J2s mortality, respectively, at a $p=0.05\%$ significance level (Table 4).

The means comparison of the percentage of J2s mortality results of different fungal biocontrol agents at 48 h of incubation indicated that *T. harzianum* caused the maximum mortality (65.4%), followed by the standard carbofuran (61.8%) and then by *P. chlamydosporium* that caused 55.6% J2s mortality. The followed treatments were *A. niger* and *P. chrysogenum* causing 44.4 and 32.5% RKN J2s mortality, respectively, at a $p=0.05\%$ significance level (Table 5).

The means comparison of the percentage of J2s mortality results of different fungal biocontrol agents at 72 h of incubation indicated that *T. harzianum* caused the maximum mortality (72.4%), followed by the standard carbofuran (68.1%) and then by *P. chlamydosporium* that caused 61.4% J2s mortality. The followed treatments were *A. niger* and *P. chrysogenum* causing 47.3 and 40.0% RKN J2s mortality, respectively, at a $p=0.05\%$ significance level (Table 6).

Table 6 Percentages of J2 mortality by various fungal biocontrol agents at varying concentrations at 72 h

Treatments	1:1	1:10	1:100	Mean (%)
Trichoderma harzianum	81.44%	71.75%	61.65%	72.46% a
Pochonia. chlamydosporium	69.28%	60.62%	50.72%	61.40% c
Aspergillus niger	55.88%	45.57%	35.67%	47.33% d
Penicillum chrysogenum	48.66%	39.59%	26.39%	40.06% e
Carbofuran(positive control)	77.32%	67.84%	56.29%	68.13% b
Control(negative control)	3.40%	3.00%	3.00%	3.13% f
Mean (%)	56.83% a	49.13% b	40.30% c	

Each value is the mean of 5 Replications. A letter followed by the same alphabet doesn't differ significantly

Greenhouse experiment

The *in-planta* results about number of galls revealed that control (negative) had the most number of galls (206.8), followed by *P. chrysogenum* having 75.1 galls per root system. *T. harzianum*-treated plants had the least number of galls 22.2 (Figs. 3, 4). All the treatments significantly lowered the RKN parameters, *i.e.,* egg masses and swollen females. *T. harzianum* had the least number of egg masses (35.7), followed by the carbofuran (standard) having 40.0 egg masses. The followed treatment was *P. chlamydosporium* (57.1). Maximum no. of egg masses was found in the check (control negative) (224.1) per root system (Fig. 3). Likewise, the control negative had a maximum no of adult females (182.7), while the least swollen females were found in *T. harzianum* and carbofuran (standard) having 21.1 and 22.75 swollen females per root system, respectively (Fig. 3).

Data regarding the eggplant growth parameters revealed that different fungal biocontrol agents significantly enhanced the eggplant growth condition over the check (negative) at $p=0.05\%$ (Fig. 5). The highest shoot weight (fresh) was achieved in *T. harzianum*-treated plants (29.8 g), followed by carbofuran (standard) (22.7 g) and *P. chlamydosporium* having 18.7 g fresh shoot weight at $p=0.001\%$ significance (Fig. 4). The highest shoot weight (dry) was recorded in *T. harzianum* treated plants (19.1 g), followed by carbofuran (standard) (15.3 g) and *P. chlamydosporium* having 11.8 g fresh shoot weight at $p=0.001\%$ significance (Fig. 5). Maximum shoot length was recorded in case of *T. harzianum*-treated plants (41.3 cm), followed by *P. chlamydosporium* (34.8 cm). All the treatments had significantly high shoot length over check (control negative) (11.8 g fresh shoot weight at $p=0.001\%$ significance) (Fig. 5). Likewise, all the treatment had significantly raised the root length than the control (negative) (Fig. 5).

Discussion

Root-knot nematode (RKN) is a small worm that is responsible for causing substantial damage to a variety of plant species including vegetables, cash crops, and ornamental plants. Management of RKNs is extremely

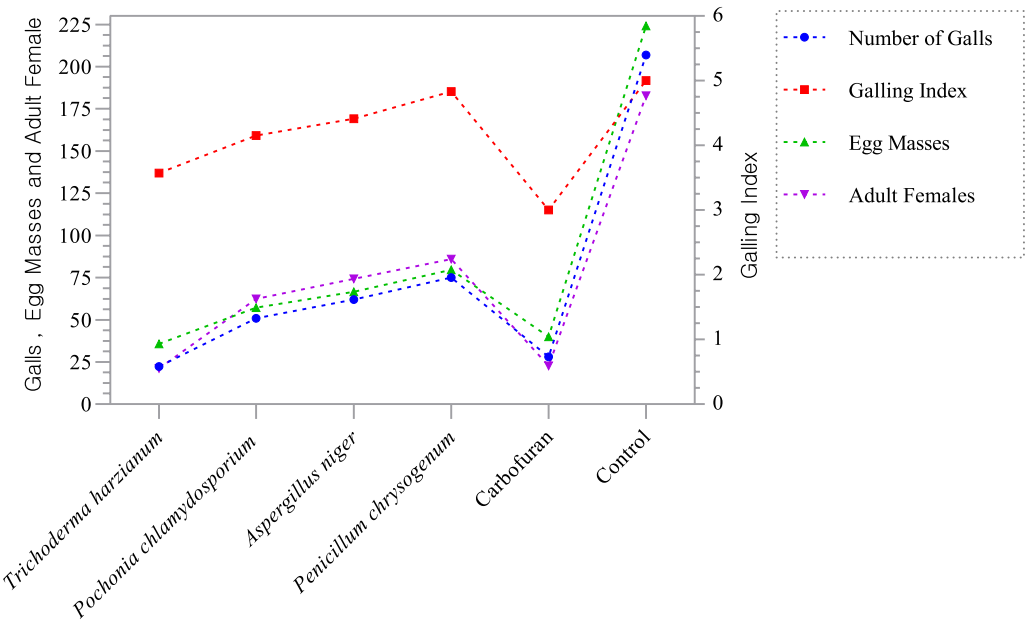

Fig. 3 Effects on the *Meloidogyne incognita* parameters in the eggplant root system inoculated with various fungal Biocontrol agents at the *in-planta* conditions

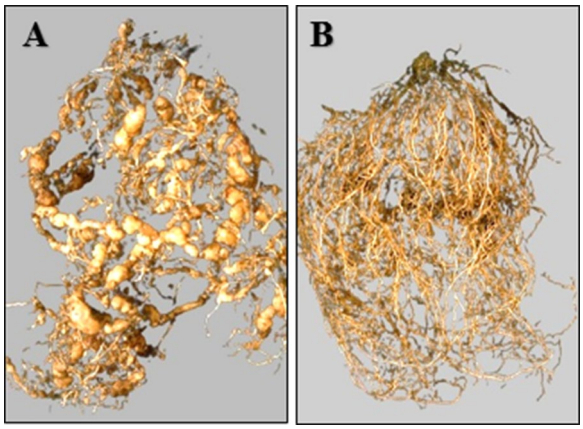

Fig. 4 Galled roots of **A** Control negative and **B** Best treatment (*Trichoderma harzianum*)

disputing because of its concealed below-ground activities. Farmers opt for chemical control, but due to its percolation into the water table, its application is useless mostly. Thus, biological control research was carried out to explore the most reliable and long-lasting control method of RKN in eggplant.

The *in vitro* results revealed that the concentrations of 1:1, 1:10, and 1:100 of the fungal inoculum successfully inhibited RKN eggs. It was also found statistically effective against the J2s mortality. The 1:1 concentration was highly effective against egg hatch inhibition and J2s mortality, followed by 1:10 and 1:100. Uddin et al (2019) reported that nematicidal efficacy increases with the increasing concentration. The order of treatment's efficacy was *T. harzianum* > Carbofuran (Control positive) > *P. chlamydosporium* > *A. niger* > *P. chrysogenum* > Control (negative) against the RKN eggs and J2s. The current best performance of *T. harzianum* against the RKN eggs and J2s was also reported by Khan et al (2022a, b) who reported that *T. harzianum* provides significant management against the RKN in *in-vitro* conditions. The *T. harzianum* conidia gather around the egg masses of the RKN, and thus, there is a possibility of breaking the defense mechanism of geletenious-matrix and leading to the ill effects on the eggs of RKN. *P. chlamydosporium* effective results indicated that it produced appressoria that could have parasitized the RKN eggs in *in vitro* bioassay. Similar findings are also reported by Bouchagier (2018), who stated that *P. chlamydosporium* alone was effective against the egg inhibition of the RKN. Literature suggested that *A. niger* produces oxalic acid and nitric acid that parasitizes the RKN eggs and J2s. However, its less effective influence of it could be due to the less exposure time (72 h of incubation) (Jang et al. 2016).

The *in-planta* studies were carried out to check the influence of nematicidal activities of the fungal biocontrol agents against the *M. incognita* and their effects on eggplant growth parameters. The findings revealed that *T. harzianum,* followed by *P. chlamydosproium*, remained persistent by efficiently lowering the RKN growth parameters, *i.e.,* less no. of eggs, swollen females and less GI. These findings are in line with the previous results of Balardin et al. (2021) who reported that *Trichoderma* efficiently controlled at the glasshouse conditions the growth parameters of RKN and inclining the growth

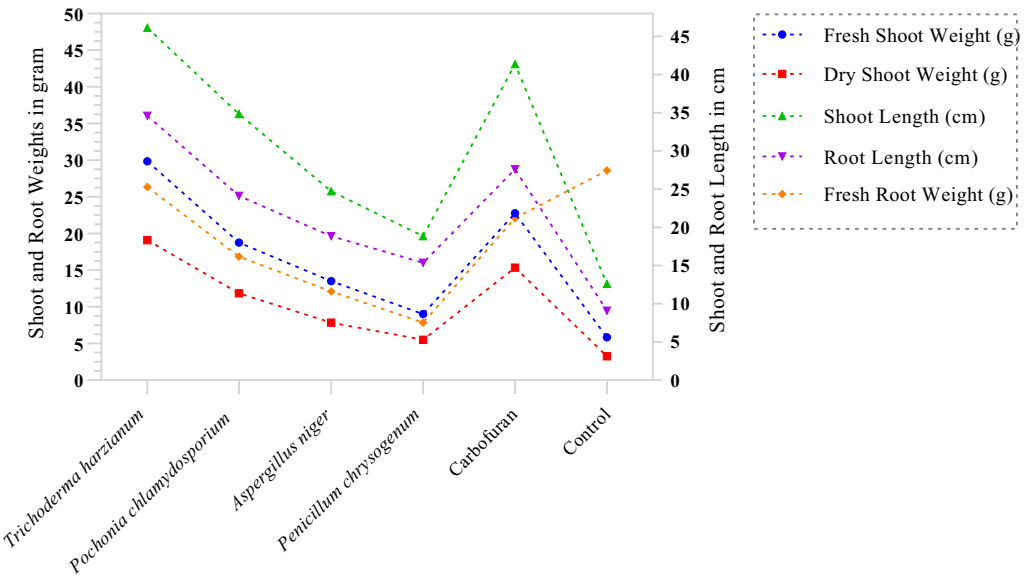

Fig. 5 Effects on the eggplant growth parameters after the addition of various fungal biocontrol agents inoculated with *Meloidogyne incognita* at the *in-planta* conditions

parameters including the yield of tomato and eggplant. The standard (Carforburan) used in the present experiment results revealed that only *T. harzianum* was better than the standard, while slightly better results were exhibited by the standard against the *P. chlamydosproium* by improving the plant growth parameters and declining the RKN productivity and stability. These results are per the results El-Nagdi et al (2019), who reported that the performance of *T. viride* and *T. harazianum* was better than Carbofuran and other applied treatments. *Trichoderma* produces an elevated amount of protease and chitinolytic enzymes which could have been parasitized eggs of nematodes (Khan et al. 2022b). The reduction in nematode eggs could have ultimately resulted in a reduction in the final population of the nematodes in the soil. *Trichoderma* also possesses a special organ called appressoria through which it can press its prey and thus, creating puncture holes and that's how hyphae enter. The mortality of egg mass could be due to the parasitization that occurred by *Trichoderma* on egg masses. It is also evident that *Trichoderma* also produces several lytic enzymes like proteases, lipases, and glucanases for the cell wall breaking (Tyskiewicz et al. 2022). *P. chlamydosproium* is thought to be an egg parasite. *P. chlamydosporia* produces branched mycelial structures (Finetti-Sialer and Manzanilla-López. 2022). According to Dallemole-Giaretta et al. (2012), its efficiency is depending on two factors, temperature and the larval stage of the embryo within the egg. Most importantly in a biological control system is the establishment of biocontrol agents in the rhizosphere, for further interaction with the plant or nematode. The nutritional values and organic fertility of the soil can shackle this establishment, and ultimately, root colonization will not occur. Egg shells of RKNs are penetrated by penetration special organ appressoria and thus disintegrate chitin of the eggs (Siddiqui et al. 2004). The chemical activity of enzymes is considered very important in causing infection. Literature suggested that infection could not be possible without the combination and presence of proteases and chitinases. This combination is considered necessary to initiate infection. *Aspergillus* species commonly occur in soils in warmer climates, in compost, decaying plant material, and stored grains, and many of them are known to produce a variety of secondary metabolites. Some *Aspergillus* species have also been reported for their biocontrol potential against root-knot nematodes (He et al. 2020).

The cultural filtrate produced in a bioreactor lowered the viability of J2s of *M. incognita* in a concentration-dependent manner. Oxalic acid is known to be the key metabolite of *A. niger* and is responsible for J2s mortality (Sikandar et al. 2020). *A. niger* is imputed to its parasitic nature and production of nematicidal serine proteases, which ultimately destroy eggshell and check egg hatching. The juvenile cuticle is mainly composed of proteins that could be degraded by the proteolytic activities of fungi (Hussain et al. 2017). Some fungi have spiny structures or other trapping devices that could facilitate the mechanical penetration of eggs as well as J2s. Eggs have surface binding components in a gelatinous matrix, which might facilitate fungal spore attachment, germination, and penetration (Gamalero and Glick 2020).

Conclusions

The fungal biocontrol agents colonizing the eggplant roots and the myco-metabolites may suppress the growth and production of pathogens including *M. incognita* and also upraise the plant growth. The present findings expressed that fungal biocontrol agents have hazardous repercussions against the egg mass, J2 and adult females of the *M. incognita*. It was concluded *T. harzianum* and *P. chlamydosporium* were very effective against RKN and could be utilized as a substitute for toxic commercially available nematicides.

Abbreviations

RKN	Root-knot nematode
J2	Second-stage juveniles
SDW	Simple distilled water

Acknowledgements

Authors would like to thank The University of Agriculture Peshawar Pakistan, which supported experimental facilities to carry out experiments.

Author contributions

JU conceived research and wrote the manuscript. FU and IN supervised experiments. SA and AURS analyzed the virulence assays. SSK and MS were responsible for writing reviews and edits. All authors read and approved the final manuscript.

Funding

This research received no external funding.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate

This article does not contain any studies with human participants or animals performed by any of the authors. All participants have given oral informed consent.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Received: 26 September 2022 Accepted: 2 February 2023
Published online: 14 February 2023

References

Alam I, Salimullah M (2021) Genetic engineering of eggplant (*Solanum melongena* L.): progress, controversy and potential. Horticulturae 7:78

Balardin RR, Bellé C, Dalla, Nora D, Ramos RF, Rodrigues JCV, Antoniolli ZI (2021) Molecular Characterization and Pathogenicity of *Trichoderma* Isolates to *Meloidogyne javanica*. In: Cristiano, Tiago EK (ed) Nematodes—recent advances, management and new perspectives. IntechOpen, London

Barker KR, Hussey RS (1976) Histopathology of nodular tissues of legumes infected with certain nematodes. Phytopathology 66(7):851–855

Bouchagier P (2018) Survival of Root-Knot nematodes and their egg-parasitic fungus *Pochonia chlamydosporia* (Goddard) on weed roots. J Plant Sci 2(2):95–105

Chen Y, Long H, Jin T, Peng Z, Sun Y, Feng T (2023) Potential of entomopathogenic nematode HbSD as a candidate biocontrol agent against Spodoptera frugiperda. InSects 14(1):2

Dallemole-Giaretta R, Freitas LG, Lopes EA, Pereira OL, Zooca RJ, Ferraz S (2012) Screening of *Pochonia chlamydosporia* Brazilian isolates as biocontrol agents of *Meloidogyne javanica*. Crop Prot 42:102–107

Darwesh OM, El-Hawary AS, El Kelany US, El-Sherbiny GM (2019) Nematicidal activity of thermostable alkaline protease produced by *Saccharomonospora viridis* strain Hw G550. Biotechnol Reptr 24:e00386

Dennis MW (2022) Soil sterilization eliminates beneficial microbes that provide natural pest suppression ecosystem services against *Radopholus similis* and *Fusarium Oxysporum* V5w2 in the endosphere and rhizosphere of tissue culture banana plants. Front Sust Food Syst 6:688194

El Aimani A, Houari A, Laasli SE, Mentag R, Iraqi D, Diria G, Khayi S, Lahlali R, Dababat AA, Mokrini F (2022) Antagonistic potential of Moroccan entomopathogenic nematodes against root-knot nematodes, *Meloidogyne javanica* on tomato under greenhouse conditions. Sci Reptr 12(1):1–9

Elkelany US, El-Mougy NS, Abdel-Kader MM (2020) Management of root-knot nematode Meloidogyne incognita of eggplant using some growth-promoting rhizobacteria and chitosan under greenhouse conditions. Egypt J Biol Pest Control 30(1):1–7

El-Nagdi WM, Youssef MM, Abd El-Khair H, Abd-Elgawad MM (2019) Effect of certain organic amendments and Trichoderma species on the root-knot nematode, Meloidogyne incognita, infecting pea (*Pisum sativum* L.) plants. Egypt J Biol Pest Control 29(1):1–9

Fatima S, Khan F, Asif M, Alotaibi SS, Islam K, Shariq M, Khan A, Ikram M, Ahmad F, Khan TA, Meena R (2022) Root-Knot disease suppression in eggplant based on three growth ages of *Ganoderma lucidum*. Microorganisms 10(5):1068

Finetti-Sialer MM, Manzanilla-López RH (2022) Exploring anastomosis of hyphae and mating-type compatibility of *Pochonia chlamydosporia* Isolates of the *Meloidogyne*. Heterodera Globodera Biotypes Pathogens 11(6):619

Fuchs GJ, Faruque ASG, Khan M, Khan AI, Malek A, Nahar K (2002) Impact of the HKI home gardening programme on VA status in Bangladesh. J Nurt 132(9):2972S-2972S

Gamalero E, Glick BR (2020) The use of plant growth-promoting bacteria to prevent nematode damage to plants. Biology 9(11):381

Giri B, Rawat R, Saxena G, Manchanda P, Wu QS, Sharma A (2022) Effect of *Rhizoglomus fasciculatum* and *Paecilomyces lilacinus* in the biocontrol of root-knot nematode, *Meloidogyne incognita* in *Capsicum annuum* L. Commun Integr Biol 15(1):75–87

He Q, Wang D, Li B, Maqsood A, Wu H (2020) Nematicidal evaluation and active compounds isolation of *Aspergillus japonicus* ZW1 against root-knot nematodes *Meloidogyne incognita*. Agronomy 10(9):1222

Hussain M, Zouhar M, Ryšánek P (2017) Effects of nematophagous fungi on viability of eggs and juveniles of *Meloidogyne incognita*. J Anim Plant Sci 27(1):252–258

Jang JY, Choi YH, Shin TS, Kim TH, Shin KS, Park HW, Kim YH, Kim H, Choi GJ, Jang KS, Cha B (2016) Biological control of *Meloidogyne incognita* by *Aspergillus niger* F22 producing oxalic acid. PLoS ONE 11(6):e0156230

Khan A, Bani-Mfarrej MF, Nadeem H, Ahamad L, Hashem M, Alamri S, Gupta R, Ahmad F (2022b) *Trichoderma virens* mitigates the root-knot disease progression in the chickpea plant. Acta Agriculturae Scandinavica Section B Soil Plant Sci 72(1):775–787

Khan A, Ahmad G, Haris M, Khan AA (2022a) Bio-organics management: novel strategies to manage root-knot nematode, *Meloidogyne incognita* pest of vegetable crops. *Gesunde Pflanzen*, pp 1–17. https://doi.org/10.1007/s10343-022-00679-2

Naz I, Saifullah P-R, Blok V, Ahmad M, Ali S (2013) Species identification of root-knot nematodes in Pakistan by random amplified polymorphic DNA (RAPD-PCR). Sarhad J Agric 29(1):71–78

Niu Y, Xiao L, De-Almeida-Engler J, Gheysen G, Peng D, Xiao X, Huang W, Wang G, Xiao Y (2022) Morphological characterization reveals new insights into giant cell development of *Meloidogyne graminicola* on rice. Planta 255(3):1–11

Oyetunde AK, Afolami SO, Kulakow P, Coyne D (2022) The differential impact of four tropical species of root-knot nematodes (*Meloidogyne* spp.) on biofortified cassava. Nematology 1:1–11

Rajan N, Debnath S, Pandey B, Singh AK, Singh RK, Singh AK, Dugbakie BN (2022) Elucidation of nature of gene action and estimation of combining ability effects for fruit yield improvement and yield attributing traits in brinjal landraces. J Food Qual. https://doi.org/10.1155/2022/8471202

Saad AM, Salem HM, El-Tahan AM, El-Saadony MT, Alotaibi SS, El-Shehawi AM, Abd-El-Mageed TA, Taha AE, Alkahtani MA, Ahmed AE, Swelum AA (2022) Biological control: an effective approach against nematodes using black pepper plants (*Piper nigrum* L.). Saudi J Biol Sci 29(4):2047–2055

Sasser JN, Eisenback JD, Carter CC, Triantaphyllou AC (1983) The international *Meloidogyne* project-its goals and accomplishments. Annu Rev Phytopathol 21(1):271–288

Siddiqui IA, Shaukat SS, Khan A (2004) Differential impact of some *Aspergillus* species on *Meloidogyne javanica* biocontrol by *Pseudomonas fluorescens* strain CHA0. Lett Appl Microbiol 39(1):74–83

Sikandar A, Zhang M, Wang Y, Zhu X, Liu X, Fan H, Xuan Y, Chen L, Duan Y (2020) In vitro evaluation of *Penicillium chrysogenum* Snef1216 against *Meloidogyne incognita* (root-knot nematode). Sci Reptr 10(1):1–9

Taylor AL, Sasser JN (1978) Identification and control of root-knot nematodes (*Meloidogyne* spp.) crop. Publ. Dep. Plant Pathology, North Carolina State University and U.S. Agency Int. Dev. Raliegh, N.C, pp 111

Tyśkiewicz R, Nowak A, Ozimek E, Jaroszuk-Ściseł J (2022) *Trichoderma*: The current status of its application in agriculture for the biocontrol of fungal phytopathogens and stimulation of plant growth. Int J Mol Sci 23(4):2329

Uddin M, Saifullah AM, Khan W, Khan B (2019) Evaluation of *Pochonia chlamydosporia* (Goddard) isolates for suppression of *Meloidogyne incognita*, root-knot nematode of tomato. J Agric Sci 11(5):70–81

Yu Y, Gui Y, Li Z, Jiang C, Guo J, Niu D (2022) Induced systemic resistance for improving plant immunity by beneficial microbes. Plants 11(3):386

Publisher's Note