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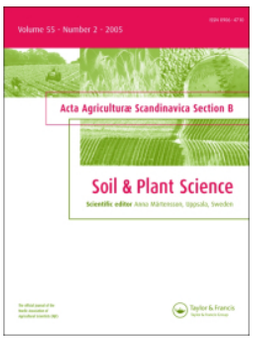

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RESEARCH ARTICLE

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Trichoderma virens mitigates the root-knot disease progression in the chickpea plant

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ABSTRACT

This study was planned to investigate the efficacy of various concentrations of *Trichoderma virens* against *Meloidogyne incognita* in vitro. The five concentrations viz., S, S/2, S/ 10, S/25, S/50 were prepared and planned for in vitro study to test the potential of T. virens against hatching and mortality of second-staged juveniles of *M. incognita*. It was observed a reduction in second-staged juveniles hatching within all tested aqueous concentrations of *T. virens*. The second-stage juvenile mortality was also recorded in the above-given concentrations of *T. virens*. The maximum decrease in second-stage juveniles hatching was found in standard aqueous fungal concentration (S). Moreover, in the same *T. virens* concentration (S), mortality of juveniles was also recorded as highest, and was followed by S/2, S/10, S/25 and S/50. Additionally, the application of *T. virens* as an individual, simultaneous, and sequential order with *M. incognita* was also investigated in pot-grown chickpea plants and found that its use was significantly effective in suppressing root-galling disease and improved the plants' growth and physiological attributes. According to the correlation coefficient analysis, the root-knot index correlated significantly with the per cent reduction of the plants' growth and physiological attributes.

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Fungal strain; root-galling disease; Meloidogyne species; pulse crop; management

Introduction

Chickpea, also known as chana or gram (Cicer arietinum L.), is a legume crop of the family *Fabaceae* and an important pulse crop in India. The rank of chickpea is listed as the world's third most productive pulse crop after peas and beans, with about 75% of its production alone coming from India (Khan et al. 2014). Chickpea is susceptible to many endo and ectoparasitic nematodes, including Meloidogyne species (Ali and Askary 2001) and Heterodera species (Sharma et al. 1999). In India, Meloidogyne species, preferably M. incognita and M. javanica, have been noted to cause an estimated loss in chickpea productivity up to 19–40% and 24–61%, respectively (Ali et al. 2010). The root-knot nematode (RKN), M. incognita, is a sedentary endo-parasite and one of the key damaging pests in the agriculture farming system, attacking crops, including pulse, cereals and vegetables. They invade the plant roots at the elongation zone and move to the vascular cylinder region, where they initiate the formation of the gall, which results in the deformation of the vascular tissues (Fuller et al. 2008). It was estimated that nematodes are responsible for the economic loss of 100 billion dollars annually (Coyne et al. 2018).

Due to their extensive host variety, short generation period, and high reproduction potential, their management is difficult (Trudqill and Blok 2001) and is recognised as a severe threat to agricultural production worldwide (Jayasinghe et al. 2003; Eyal et al. 2006). Concerning economic losses produced by RKNs, several strategies have been undertaken for nematode control in agriculture, including applying chemical nematicides. Chemical nematicides are the most reliable management option against plant-parasitic nematodes (PPNs). In recent years, chemicals like 1,3-dichloropropene (1,3-D) and metam sodium have been utilised as alternatives to methyl bromide (Desaeger et al. 2017). However, compounds like metam sodium can have adverse ecological and human health effects. Pruett et al. (2001) suggest that metam sodium can induce both allergic dermatitis and/or asthma in humans. It is, therefore, recently being phased out because of the high negative

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impact on human and animal health. Consequently, the search for sustainable alternatives to chemical nematicides has become of paramount interest for research. One of the most promising RKNs management strategies is biological control through the action of living organisms defined as biocontrol agents (BCAs). BCAs can directly act as antagonists through antibiosis and competition mechanisms for the nutrients or space or indirectly as inducers of resistance by activating the plant immune system (Molinari and Leonetti 2019; Poveda et al. 2020).

Presently, there is a large availability of commercial BCAs formulates, when opportunely used, which have most recently shown suitable performances against RKNs (Molinari and Leonetti 2019; Pocurull et al. 2020). Disease-suppressing microbes can secrete toxic compounds which inhibit plant pathogens and show toxicity to eggs or juveniles of *Meloidogyne* spp. (Albehadeli et al. 2019)**.** However, fungal biocontrol agents such as Trichoderma spp. have remarkable applications against RKNs (de Medeiros et al. 2017). The efficiency of BCAs depends on the types of nematode species, specificity to host plants, and their root exudates (Singh and Mathur 2010). Antagonistic microorganisms e.g. Trichoderma harzianum, Trichoderma viride, Pasteuria penetrans, Purpureocillium lilacinum, Bacillus subtilis, and Pochonia chlamydosporia have been identified as potential BCAs for management of RKNs (Huang et al. 2016; Mukhtar 2018; Ghahremani et al. 2019). Few metabolites with nematicidal properties have been isolated from *Trichoderma* spp.; some of these are acetic acid, gliotoxin, trichorzianine, viridin (Li et al. 2007), trichodermin (Zhou et al. 2018), and cyclonerodiol (Shi et al. 2020). The nematicidal potential of *Trichoderma* spp. are increasingly being harnessed to develop new and safer biocontrol agents against parasitic nematodes such as *Globodera pallida*, *Heterodera avenae*, *M. incognita*, *M. javanica*, *M. hapla*, and *Pratylenchus brachyurus* (Braithwaite et al. 2016; Tariq-Javeed et al. 2021). *Trichoderma* spp. that have lethal effects against *Meloidogyne* species include *T. harzianum*, *T. koningii*, *T. longibrachiatum*, *T. citrinoviride* and *T. viride* against *M. incognita* (Fan et al. 2020; Khan et al. 2020); *T. hamatum*, *T. harzianum*, T. *koningii*, *T. koningiopsis*, and *T. viride* against *M. javanica* (Qureshi et al. 2012; Elgorban et al. 2014); *T. asperellum*, *T. harzianum*, and *T. viride* on *M. hapla* (Braithwaite et al. 2016). Therefore, in this research work, a set of experiments was conducted to determine: (i) antagonistic potential of Trichoderma *virens* for infective second-stage juveniles (J2s) of *M. incognita* using mortality and J2s hatching inhibition test; (ii) efficacy of T. *virens* exposed to J2s inoculated chickpea roots under pots study.

Materials and methods

Materials

Chickpea, *Cicer arietinum* L. cv. Avrodhi was tested as a host cultivar. Seeds were surface sterilised with Sodium hypochlorite (NaOCl) (0.1% v/v) for 15 min, then were rinsed three times with sterile distilled water (DW) for five minutes. The pure culture of the fungus strain, *Trichoderma virens* (ITCC-7351), was obtained from the Indian Agricultural Research Institute, New Delhi, India. The fungus strain is sub-cultured on Potato Dextrose Agar (PDA) medium. The mass multiplication of *T.virens* was done by using Richards Medium.

Nematode inoculum and maintenance

The RKN, *M. incognita,* was maintained on eggplant grown in a glasshouse. For J2s collection, egg masses detached from the infected roots (Hussey and Barker 1973) and kept in sterile water for hatching within a Biological Oxygen Demand (BOD) Incubator (28 ± 2°C) for four days to allow the hatching process. After four days, hatched J2s were collected. These freshly hatched J2s were considered nematode inoculum for this study.

Meloidogyne species identification using Scanning Electron Microscopy (SEM)

Species identification was performed using SEM analysis and characterised based on perineal pattern features (Sasser and Carter 1982). A mature female of *M. incognita* was separated from the infected eggplant root. The proposed method for preparing perineal patterns was followed (Abrantes and Santos 1989). The perineal pattern was coated with 14 nm gold, and images were captured using SEM (JSM 6510 LV Jeol-Japan). The morphology of the perineal pattern was studied to characterise the RKN species (Figure 1). The angularly oval structure with a high dorsal arch in a typical pyriform was seen, and striations were in distinct waves that bent towards lateral lines without interruption. These striations were straight with an oval appearance in the ventral regions. The above-obtained features of the perineal pattern confirm the RKN species, *M. incognita*.

Cultural filtrate preparation of T. virens

For the mass production of *T. virens,* Richards's medium was utilised (Riker and Riker 1936). The fungal mycelia mat on filter paper was washed in sterile water, and extra water and nutrients were removed with the

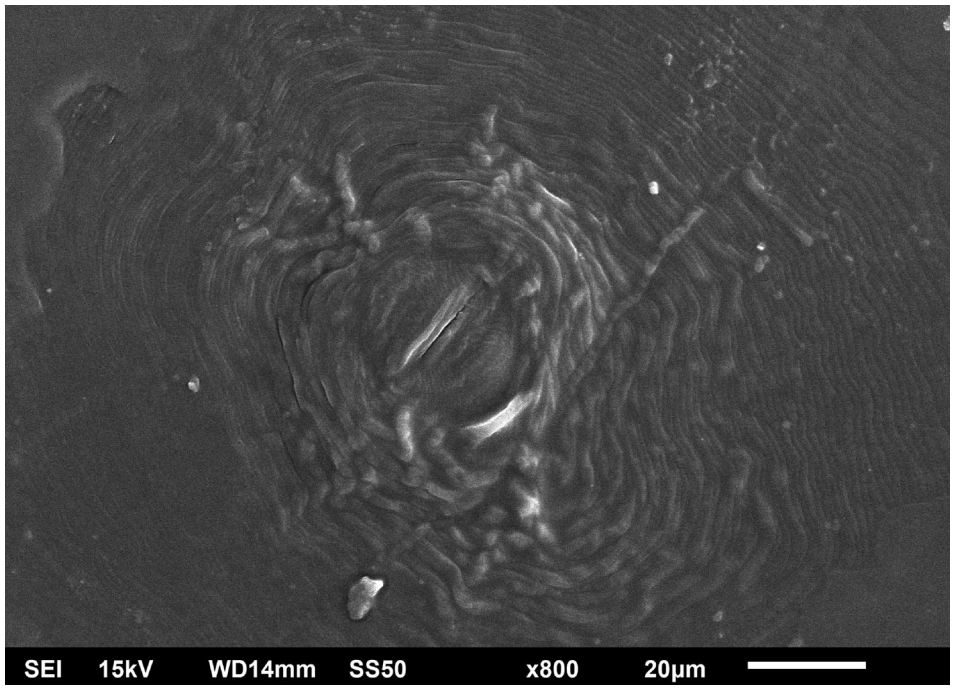

Figure 1. Scanning electron microscopy showing the perineal pattern of *M. incognita*. The high squared dorsal arch and wavy striae are key features of *M. incognita*.

help of blotting paper. Ten grams of mycelia mat (fungal inoculum) were mixed in 100 mL of DW followed by blending in a waring blender (10,000 RPM) for 30 s. The inoculum collected was labelled as Standard suspension (S), and consecutive concentrations such as S/2, S/10, S/25, S/50 were prepared using DW (Mukhtar et al. 2013). 10 mL of 'S' concentration of fungal inoculum were used to inoculate chickpea plants.

T. virens for J2s hatching test

The inhibitory effect of *T. virens* on J2s hatching of *M. incognita* was tested using different concentrations (S, S/2, S/10, S/25, S/50) through the egg mass dipping method. Four egg masses were placed into Petri dishes containing 10 mL of each prepared concentration of *T. virens*. Petri dishes were covered with parafilm to prevent evaporation and then placed in a BOD incubator (28°C). Each treatment was repeated five times, excluding control. The experiment was conducted twice under the same conditions, and the mean of the two was calculated. The hatching value was calculated by counting the number of J2s hatched per replication after four days of incubation and calculated per cent inhibition using the mentioned formula (Khan et al. 2019).

$$\text{Percent Mortality} = \frac{C0 - T\alpha}{C0} \times 100$$

Where, C_0 = Number of J2s hatched from the egg masses in DW (control), T_α = Number of J2s hatched from the egg masses in each concentration of *T. virens*.

T. virens for J2s mortality test

Similar five concentrations of *T. virens* were used to test J2s mortality of *M. incognita*. For the mortality test, 1 mL of DW containing 100 J2s was poured into Petri dishes by adding 9 mL of different concentrations of *T. virens*. Petri dishes with only water were labelled as control. Each treatment had five replications. Petri dishes were sealed with the help of a lid, wrapped in parafilm, and incubated at 28°C in BOD incubator. The dead and alive J2s were counted separately after 8, 16, and 24 h of incubation using a stereoscopic microscope. The per cent mortality of J2s was noted accordingly with the mean percentage of dead nematodes. Those J2s that looked like flexible or winding shapes were declared alive (El-Rokiek and El-Nagdi 2011), and if J2s did not move and the outline of their body appeared as straight, they were considered dead. The experiment was conducted twice under the same conditions, and the mean of the two was calculated. The per cent mortality was calculated using the following formula (Sun et al. 2006).

$$\text{Percent mortality of J2s} = 100 \times \text{Dead J2s/Total J2s}$$

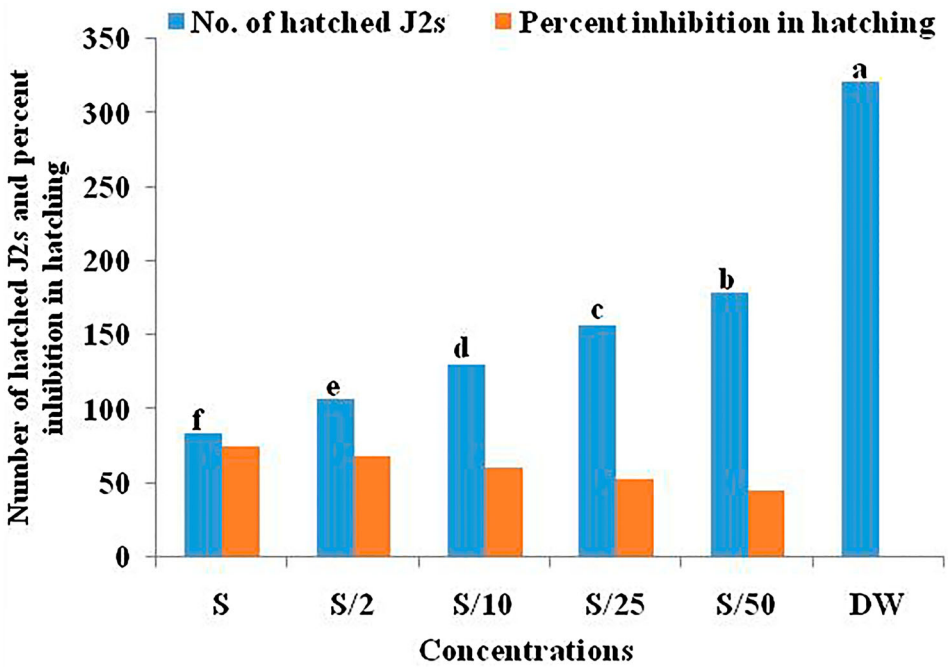

Figure 2. Effect of different concentrations of *T. virens* on J2s hatching of *M. incognita* over four days of incubation *in vitro*. DW = Distilled water.

Effect of individual, sequential, and simultaneous application of T. virens and M. incognita on chickpea

The experiment was laid out in a glasshouse. The clay pots were filled with 1 kg sterilised soil mixed with farmyard manure in a ratio of 3: 1 (sandy loam: farmyard manure). The pots were autoclaved at twenty-pound pressure at 121°C for twenty minutes. Five to seven sterilised seeds of chickpea cv. 'Avarodhi' were sown in pots. The water was sprayed through the sprinkler when necessary in pots for germination. When the seedlings grew into two sets of leaves, the plants were thinned in each pot. Healthy and stable seedlings were selected per pot, and the remaining ones were removed, including in control. The experiment was conducted with five treatment replications in a completely randomised design (CRD). 2500 hatched J2s of *M. incognita* and 'S' concentration of *T. virens* (10 mL) were inoculated around the roots of chickpea plants. However, 10 mL of DW were used in control plants *instead of T. virens* inoculum. The experiment was terminated at approximately 60 days. All the tested plants were washed in running tap water to separate soil adhered, and then assessments were performed. The experiment was conducted twice under the same conditions and the mean of the two was calculated. The plant growth and physiological and pathological parameters of chickpea were considered and presented in tables and figures.

Experimental design

The following experimental set-up was designed.

(1) Tv: Inoculated with *T.virens* alone
(2) Tv→Mi_{15}: *T.virens* treatment given 15 days prior *M. incognita* inoculation
(3) Tv + Mi: Inoculated with *T.virens* and *M. incognita* simultaneously
(4) Mi→Tv_{15}: *M. incognita* inoculated 15 days prior *T.virens* treatment
(5) Nematode only: Inoculated with *M. incognita* alone
(6) Control: No inoculation of *T.virens* and *M. incognita*

Estimation of growth, yield, and physiological attributes of chickpea

The plant growth, yield, and physiological attributes were analysed at termination. The growth attributes, including plant length, plant fresh weight, the number of pods and nodules per plant and physiological attributes, including nitrate reductase activity ($\mu mh^{-1}g^{-1}$), chlorophyll content (mg/g), and carotenoid content (mg/g) were determined following the methods described by Jaworski (1971), Mackinney (1941) and MacLachlan and Zalik (1963), respectively.

Determination of pathological parameters

The root-knot index (RKI) was determined by following a 0–5 scale (Taylor and Sasser 1978). Where, 0 indicates no

Table 1. Effect of different concentrations of T. virens on the J2s mortality of M. incognita at 8, 16 and 24 h incubation in vitro study.

Treatment	Time (hours)	Number of dead J2s (Mean ± SE) in different concentrations					
		S	S/2	S/10	S/25	S/50	DW
T. virens	8	49.40a ± 1.90 (44.90%)	38.80b ± 1.22 (35.27%)	27.40c ± 1.76 (24.90%)	18.20d ± 1.37 (16.54%)	11.20d ± 1.04 (10.18%)	0 ± 0e (0%)
	16	73.60a ± 1.76 (66.90%)	58.40b ± 1.33 (53.09%)	41.60c ± 0.99 (37.81%)	30.40d ± 1.09 (27.63%)	23.40d ± 1.09 (21.27%)	0 ± 0e (0%)
	24	92a ± 1.66 (83.63%)	75.60b ± 1.31 (68.72%)	64c ± 1.33 (58.18%)	52.60d ± 1.31 (47.81%)	42.60e ± 0.99 (38.72%)	0 ± 0f (0%)

Each value is the mean of two trials with five replicates of each.
DW: Double water (control); S, S/2, S/10, S/25, S/50: Concentrations of *T. virens*; S: Standard concentration of *T. virens*; SE: Standard Error; J2s: Second-stage juveniles.
Values are given in parentheses represent the per cent J2s mortality over control.
Values are given without parentheses represent the number of the dead J2s of *M. incognita*.
The different letters (a, b, c, d, e) represented in the table are significantly different at $p = 0.05$ using Duncan's Multiple Range Test (DMRT).

galls/root, 1 indicates 1–2 galls/root, 2 indicates 3–10 galls/root, 3 indicates 11–30 galls/root, 4 indicates 31–100 galls/root, and 5 indicates ≥100 galls/root. At harvesting time, the estimation of the final population of J2s per 250 g of soil was determined by Cobb's sieving and decanting method (Cobb 1918), followed by modified Baermann's funnel technique (Southey 1986).

Statistical analysis

The data analysis was performed by applying R software (2.14.1). The Duncan's Multiple Range Test (DMRT) were calculated at $p = 0.05$ to show the significant differences between the treatments. However, the principal component analysis (PCA) showed the variability among studied attributes by using Origin software [version 2019b (9.65)]. The coefficient of correlation was determined by using Microsoft excel.

Results

T. virens for J2s hatching and mortality test

T. virens significantly inhibited the J2s hatching and found that all prepared concentrations (S, S/2, S/10, S/25, and S/50) of the fungal strain potentially inhibit J2s hatching of *M. incognita*. The per cent inhibition was maximum in standard concentration (S), followed by S/10, S/25, and S/50, respectively (Figure 2). It was also found that inhibition in J2s hatching was directly proportional to the strength of fungal concentration during the hatching test.

In the mortality test, J2s were exposed to different concentrations of *T. virens* viz., S, S/2, S/10, S/25, and S/50. The J2s mortality was analysed in each treatment after 8, 16, and 24 h of incubation. The per cent mortality of J2s was 83.63% in standard concentration (S) at 24 h of incubation (Table 1). The S/2, S/10, S/25, and S/50 concentrations also showed 68.72%, 58.18%, 47.18%, and 38.72% J2s mortality at 24 h of incubation, respectively. However, the per cent mortality of J2s decreased as the incubation time declined (Table 1). All the concentrations significantly killed the J2s compared to the control.

Effect of individual, sequential, and simultaneous application of T. virens and M. incognita on chickpea

We found a significant improvement in the growth of chickpea plants when the different sequences of treatments of *T. virens* and *M. incognita* were applied. The results revealed that different sequences of treatments modified the growth attributes of chickpeas. The application of *T. virens* alone showed the most significant

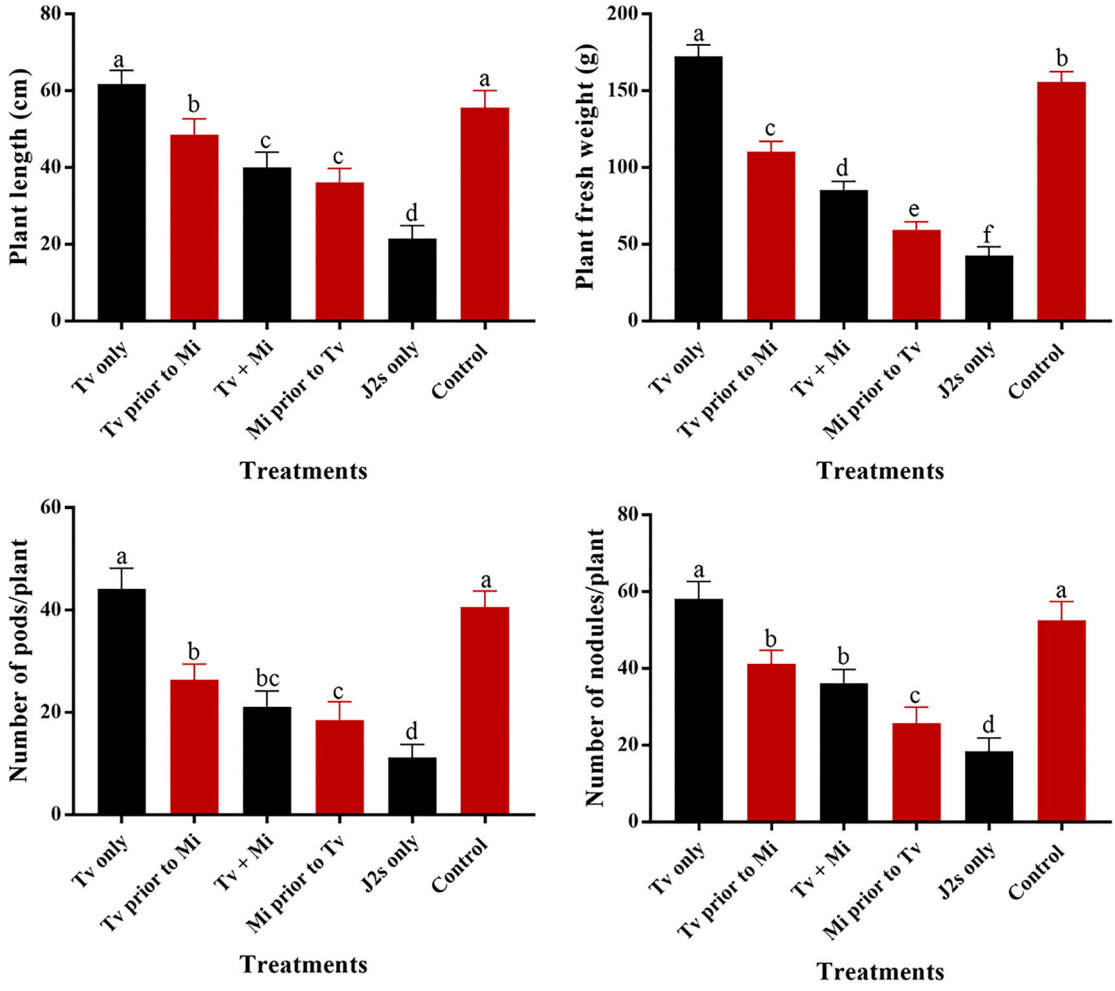

Figure 3. Effect of different treatments of *T. virens* and *M. incognita* on the growth attributes of chickpea. Tv only = *T. virens* only; Tv prior to Mi = *T. virens* treatment given 15 days prior *M. incognita*; Tv + Mi = *T. virens* and *M. incognita* given simultaneously; Mi prior to Tv = *M. incognita* inoculated 15 days prior *T. virens*; J2s only = *M. incognita* only.

($p < 0.05$) improvement in plant growth attributes. It was followed by *T. virens* when treated 15 days before *M. incognita* inoculation (Tv prior to Mi), inoculated with *T. virens* and *M. incognita* simultaneously (Mi + Tv), and *M. incognita* inoculated 15 days before *T. virens* treatment (Mi prior to Tv). The highest reduction was noticed in growth attributes when *M. incognita* was inoculated alone. The suppression in growth attributes was found in the order of (J2s only) > (Mi prior to Tv) > (Mi + Tv) > (Tv prior to Mi) > (Tv only) (Figure 3). According to the correlation coefficient analysis, the RKI is significantly correlated with plant growth attributes; including plant length, plant fresh weight, number of pods and nodules, chlorophyll and carotenoid content, and nitrate reductase activity (Figure 4). The scattered points in the graphs represent whether or not the two variables have a relationship. RKI has a strong linear relationship with plant length ($R^2 = 0.91$), plant fresh weight ($R^2 = 0.98$), number of pods ($R^2 = 0.99$), number of nodules ($R^2 = 0.95$), chlorophyll content ($R^2 = 0.94$), carotenoid content ($R^2 = 0.99$) and nitrate reductase activity ($R^2 = 0.99$). As the relation is positive, the surge in RKI increased the per cent reduction of chickpea attributes was observed (Figure 4).

The applied different sequence of treatments significantly ($p < 0.05$) enhanced the physiological parameters of chickpea plants. The application of *T. virens* alone significantly increased the chlorophyll and carotenoid content and NR (nitrate reductase) activity. It was followed by *T. virens* treatment given 15 days before *M. incognita* inoculation (Tv prior to Mi), inoculation with *T. virens* and *M. incognita* simultaneously (Mi_{15} + Tv), and *M. incognita* inoculated 15 days before *T.virens* treatment (Mi prior to Tv). However, the inoculation of *M. incognita* alone caused the highest reduction in physiological attributes. The suppression of physiological parameters was found in the order of (J2s only) > (Mi prior to Tv) > (Mi + Tv) > (Tv prior to Mi) > (Tv only) (Figure 5).

In the case of the pathogenic effect of *M. incognita,* applying different sequences of treatments

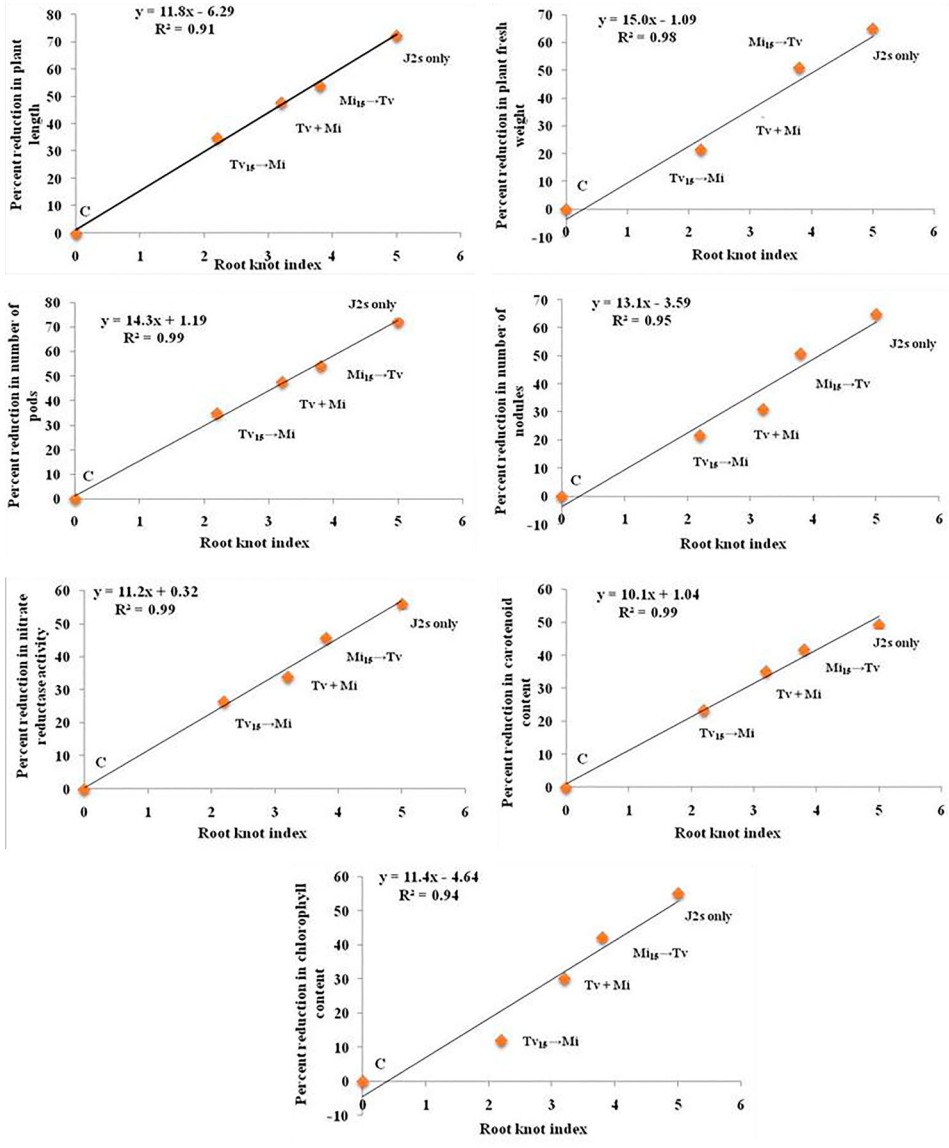

Figure 4. Relationship between the RKI and per cent reduction in various growth attributes of chickpea (C = Control; $Tv_{15} \rightarrow Mi$ = *T. virens* treatment given 15 days prior *M. incognita*; Tv + Mi = *T. virens* and *M. incognita* given simultaneously; $Mi_{15} \rightarrow Tv$ = *M. incognita* inoculated 15 days prior *T. virens*; J2s only = *M. incognita* only).

significantly ($p < 0.05$) reduced the RKI and J2s population in the soil. Application of *T. virens* treatment given 15 days before *M. incognita* inoculation (Tv prior to Mi) showed a significant reduction in the RKI and J2s population in soil followed by inoculation with *T. virens* and *M. incognita* simultaneously (Mi + Tv), and *M. incognita* inoculated 15 days prior *T. virens* treatment (Mi prior to Tv). However, J2s inoculation alone showed the highest RKI in the root system and J2s population in the soil. The efficacy of all sequence of treatments was found for reduction of RKI and J2s population in the order of (Tv prior to Mi) > (Mi + Tv) > (Mi prior to Tv) > (J2s only) (Figure 6). The results of the principal component analysis revealed that the J2s population of *M. incognita* in soil and RKI was strongly correlated with other chickpea attributes. Scatter biplot analysis revealed that different treatments of *T. virens* and *M. incognita* applied in the sequence were highly effective in reducing the pathogenic effect and enhancing chickpea growth and physiological attributes, as shown in Figure 7.

Discussion

In vitro experiment, the tested concentrations *viz*., S, S/2, S/10, S/25 and S/50 of *T. virens* were effectively inhibited J2s hatching and caused J2s mortality of *M. incognita*. Standard concentration 'S' was found to be highly effective in reducing J2s hatching and showed the

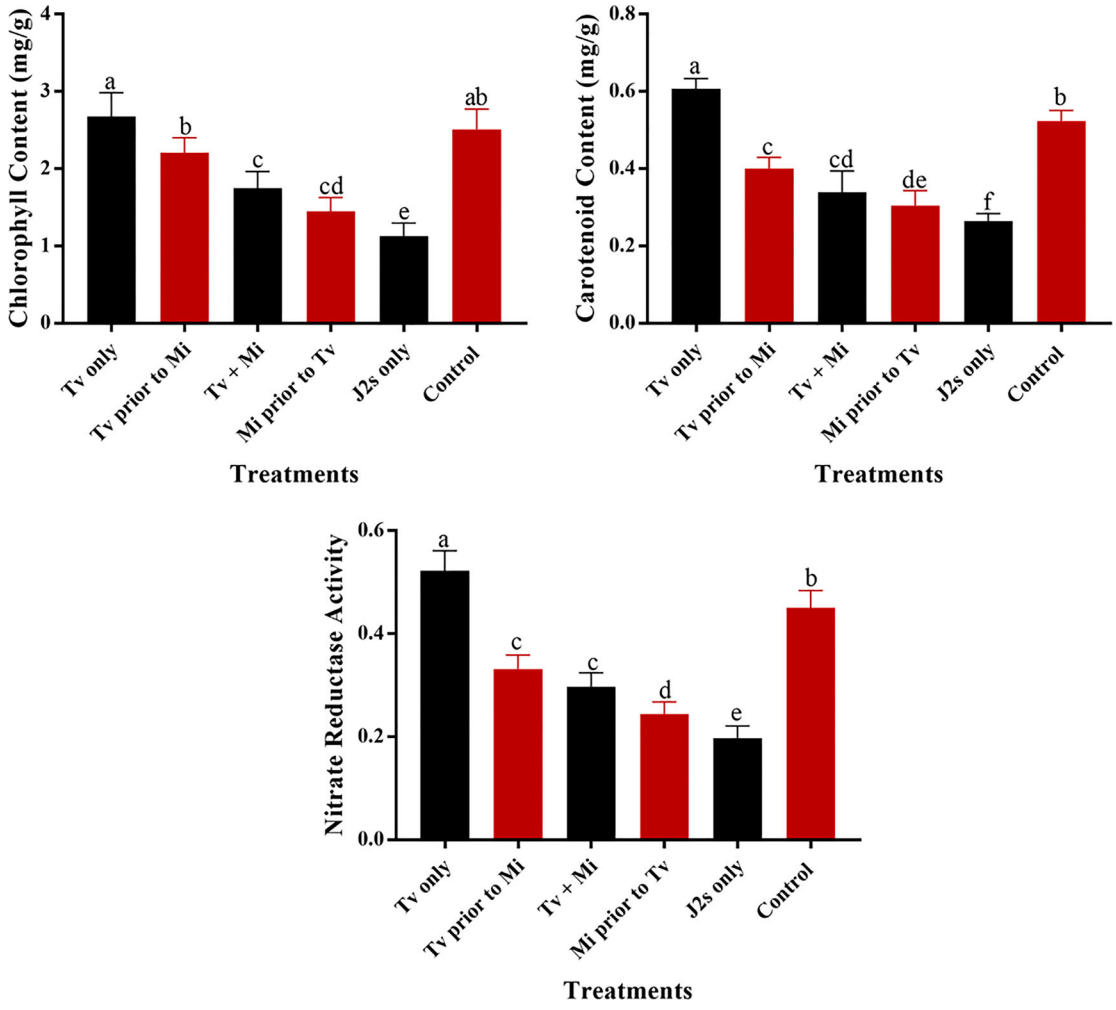

Figure 5. Effect of different treatments of *T. virens* and *M. incognita* on the physiological attributes of chickpea. Tv only = *T. virens* only; Tv prior to Mi = *T. virens* treatment given 15 days prior *M. incognita*; Tv + Mi = *T. virens* and *M. incognita* given simultaneously; Mi prior to Tv = *M. incognita* inoculated 15 days prior*T. Virens*; J2s only = *M. incognita* only.

highest toxicity towards J2s of *M. incognita*, followed by S/2, S/10, S/25, S/50 (Figure 2; Table 1). However, contrary findings were also reported by Moo-Koh et al. (2022), they reported in their study that 50% concentration of *T. virens* showed only 22% mortality of J2s of *M. incognita*. Singh and Mathur (2010) found that *T. viride* and *T. harzianum* caused mild inhibition in J2s hatching and showed least J2s mortality compared to other applied fungal BCAs. The nematicidal effect of fungal inoculum was increased when exposure time extended. Meyer et al. (2004) and Elbadri et al. (2008) reported in their study that the impact of fungal inoculum varied from concentration to concentration, thus confirming these findings. In our study, the concentration and incubation period were important factors. Sharon et al. (2001) reported that the nematicidal activity of *T. viride* is due to chitinase and protease enzymes that infect nematode larvae and eggs. Abo-Elyousr et al. (2010) noted that *Trichoderma* spp. produced chitinase enzyme in the culture that can inhibit the egg hatching of nematodes.

In the pot experiment, inoculation of *T. virens* either individually, simultaneously, or sequentially with *M. incognita* on chickpea was performed and found that all the treatments showed significant improvement in growth and physiological attributes and the reduction in pathological parameters. The highest reduction in RKI and nematode populations was found in those plants treated with *T. virens* given 15 days prior to *M. incognita*. Because, *T. virens* were got sufficient time to colonise the root system and making it less susceptible to nematode, lower penetration to J2s of *M. incognita* or released compounds which have an antagonistic effect on *M. incognita* (Figure 6). However, contrary findings were also reported by Meyer et al. (2001). According to their study, the role of *T. virens* in reducing the numbers of eggs and J2s on the root of bell pepper is comparatively less than *Burkholderia*

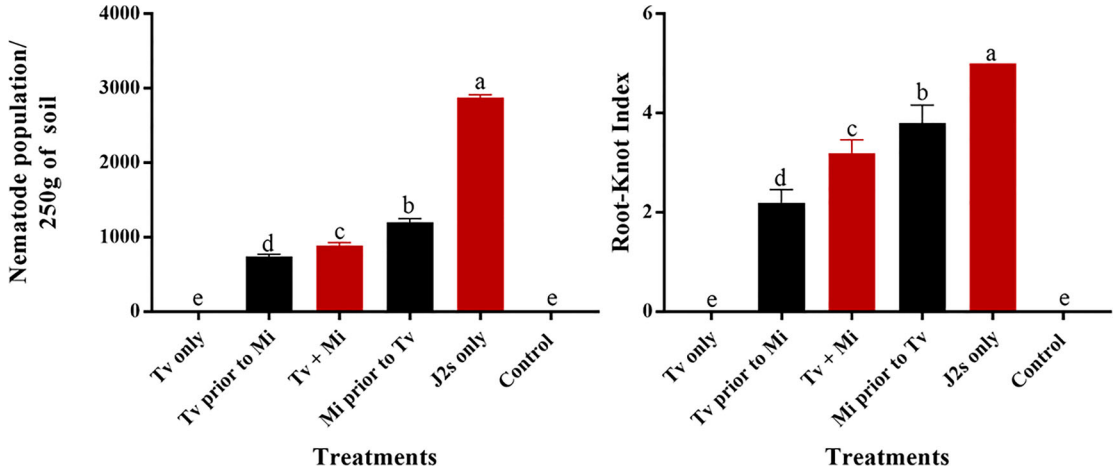

Figure 6. Effect of different treatments of *T. virens* and *M. incognita* on thepathological attributes of chickpea. Tv only = *T. virens* only; Tv prior to Mi = *T. virens* treatment given 15 days prior *M. incognita*; Tv + Mi = *T. virens* and *M. incognita* given simultaneously; Mi prior to Tv = *M. incognita* inoculated 15 days prior *T. virens*; J2s only = *M. incognita* only.

cepacia. Herrera-Parra et al. (2018) reported that among the application of four *Trichoderma* spp., plants treated with *T. virens* showed a higher number of galls per root, making it statistically lower than the other *Trichoderma* spp. used. An increment in growth attributes and reduction in the pathogenic effect of *M. incognita* could be because *T. virens* that colonise plant roots give a physical deterrent for J2s to penetrate

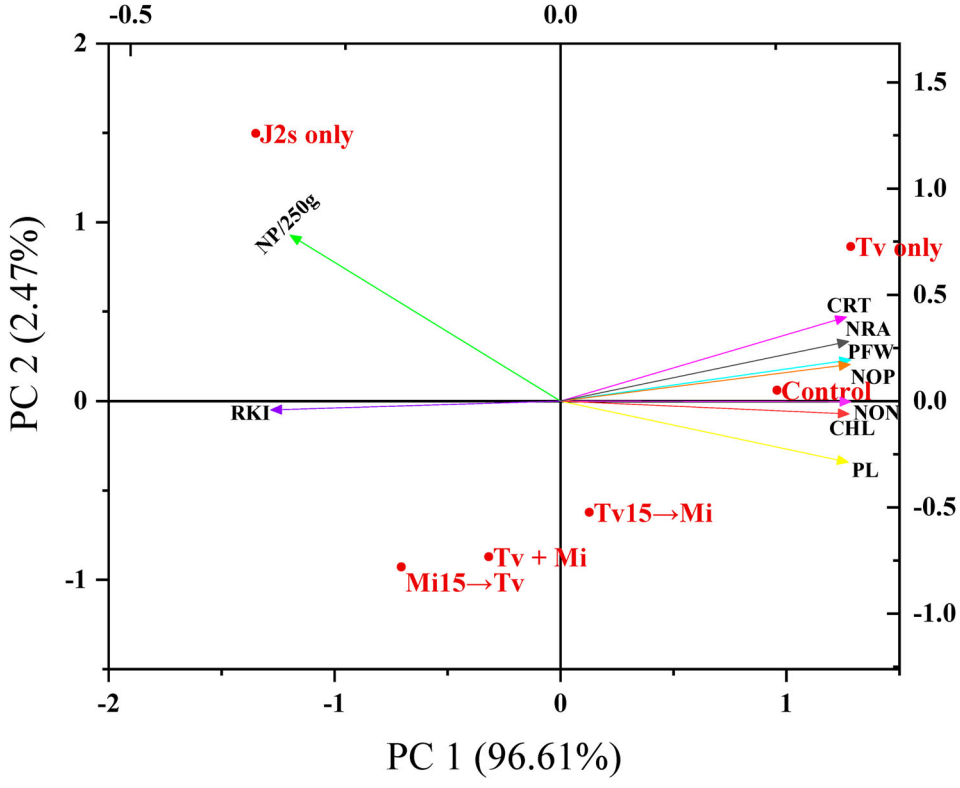

Figure 7. The biplots of principal component analysis, comparing the effects of different concentrations of *T. virens* on various studied parameters of J2s inoculated chickpea plants (PFW = Plant fresh weight; PL = Plant length; NO*P* = Number of pods; NON = Number of nodules; CHL = Chlorophyll content; CRT = Carotenoid content; NRA = Nitrate reductase activity; RKI = Root knot index; NP/250g = Nematode population in 250 g of soil; Tv only = *T. virens* only; Tv_{15} →Mi = *T. virens* treatment given 15 days prior *M. incognita*; Tv + Mi = *T. virens* and *M. incognita* given simultaneously; Mi_{15} →Tv = *M. incognita* inoculated 15 days prior*T. virens*; J2s only = *M. incognita* only).

in roots. Soil application of *T. virens* in chickpea plants minimised the population of J2s in soil due to the colonising action of *T. virens* near the roots (Figure 3). However, contrary findings were also reported by Zhang et al. (1996). They reported that *T. virens* did not suppress the reproduction of *M. incognita* on cotton. Analysis of correlation coefficient exhibited that RKI positively correlated with plant length, plant fresh weight, chlorophyll and carotenoid content, NR activity and number of pods and nodules (Figure 4). Scattered points which are existing in graphs show whether two variables have a relationship or not. Maximum scattered points with minimum correlation were found between the RKI and plant length ($R^2 = 0.91$) with positive correlation and maximum correlation with highly condensed points observed between RKI and number of pods, carotenoid content, chlorophyll content and NR activity ($R^2 = 0.99$) (Figure 4). Our finding confirmed with Rich et al. (1984), reported that significant positive correlations were observed between nematode numbers and plant yield of tobacco. The colonisation of *T. virens* may create adverse conditions for the J2s to penetrate the plant roots. In addition, it was possible that toxic secretions produced by *T. virens* may create a suppressive effect on nematodes and make the plants less susceptible to the attack of nematodes. After colonisation, toxic secretions released by the applied BCAs induced a suppressive effect on *M. incognita* and improved the atmospheric N2 accessibility to the plants (Bashan and Holguin 1997). Furthermore, *M. incognita* inoculation 15 days prior to *T. virens* showed the least improvements in growth attributes of chickpea, as firstly applied J2s had sufficient time for multiplication and caused infection in the root system of plants (Figure 3). Contrary findings were also reported by Fan et al. (2020). They reported that T. citrinoviride treated plants increased shoot length, root length, root fresh weight, and root dry weight by 15.61, 23.32, 35.08, and 33.33%, respectively, compared to those of with untreated plants. Inoculation of *T. virens* either individually, simultaneously, or sequentially with *M. incognita* showed significant improvement in physiological attributes compared to J2s only (Figure 5). However, contrary findings were also reported by Singh et al. (2017). They reported that *T. harzianum* showed the highest chlorophyll content compared to carbofuran treated plants. Multiple action mechanisms of *Trichoderma* spp. were recorded to contribute to the biological control, including competition for space and nutrients, antibiosis, myco-parasitism, and induction of systemic resistance in plants (Lombardi et al. 2018). The mechanisms of *Trichoderma* in promoting plant growth include the production of auxin-like compounds, increased availability of nutrients, affecting the root system, and inducing systemic resistance to plants (Li et al. 2015; Marra et al. 2019). The reduction in the roots galls may be due to the failure of most J2s of *M. incognita* to enter the host plant roots. The BCAs applied in the roots of host plants provide a physical barrier for the penetration of J2s of *M. incognita* and enhance root growth and nutrient uptake (Wickramaarachchi and Ranaweera 2008). *Trichoderma* spp. has increased systemic resistance to plant diseases via root colonisation which activates the plant defence mechanisms (Forghani and Hajihassani 2020). The obtained results revealed that *T. virens* have antagonistic activity for *M. incognita*. In pots-grown chickpea, *T. virens* reduced the root-galling infestation by killing the infective J2s of *M. incognita*. Thus, the potential of *T. virens* could be considered for better crop growth by reducing the disease infestation. However, the use of *T. virens* must be extended to field experiments to gather the maximum data for considering the antagonistic potential against pests and diseases. The obtained results data were based on *in vitro* and pot experiments within this study. Therefore, findings from this study have shown the potential for using *T. virens* as an ecological safe option to manage the RKNs, *M. incognita* in agricultural practices. It would also minimise the use of chemical nematicides in the farming system.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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