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RESEARCH

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Unveiling the potential of *Trichoderma harzianum* against *Heterodera cajani* in pigeon pea: impact on cysts, egg and juveniles abundance

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Abstract

Background Pigeon pea is affected by the cyst nematode *Heterodera cajani* limiting the production. *H. cajani* can be managed by biological agents such as *Trichoderma harzianum*. Thus, the determination of this experiment was to estimate native strains of *T. harzianum* isolated from pigeon pea fields in India on population of cyst, eggs and juveniles of *H. cajani*. Pots experiment was conducted at the playhouse of Sam Higginbottom University of Science and Technology, Prayagraj (UP), India, during the year of 2018–2020. Earthen pots were filled with sterilized soil @ 10 kg/pot for each replicate of the treatments. Ten days before sowing of the pigeon pea seed, about 5000 spores of *T. harzianum* were amended in to the soil for proper colonization. Fifteen days after seed germination, 200 cysts/pot were inoculated near the root zone of pigeon pea plant. The pots were irrigated when required. Observations were recorded of cyst population, eggs and second juveniles of *H. cajani*/500gm of pigeon pea rhizospheric soil at 90 days after sowing.

Results Among all the isolates of *T. harzianum*, the treatment (T 7) reduced of cyst population (12), eggs population (234) and juveniles' population (153) as compared with all other treatments including control at 90 days after sowing of pigeon pea seeds in the year 2018–2019. Similarly, in 2019–2020 reduction in cyst population (11), eggs population (189) and juveniles population (160) was observed at 90 days after sowing in *Trichoderma* isolates (T 7) as compared to control.

Conclusions The findings of this study are very relevant since seven isolates of native *T. harzianum* were promising to suppress the number of cysts in 79.31 and 83.07%, eggs in 22.00 and 38.83% and juveniles' population in 17.74 and 20.39% of the nematode *H. cajani,* besides promoting the pigeon pea plant growth.

Keywords Pigeon pea, *Heterodera cajani*, *Trichoderma harzianum*, Cyst population, Management

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Background

The pigeon pea (*Cajanus cajan* L.), which ranked sixth internationally behind peas, broad beans, lentils, chickpeas and common beans, is farmed on 5.4 million hectares of land worldwide and produces 4.49 million metric tons annually (Fatokimi and Tanimonure 2021). More than 100 pathogens attack this crop including nematodes, fungus, viruses, bacteria and MLOs (Reddy 2021). Significant damage to the crop at all stages is caused by plant parasitic nematodes which are microscopic (Maurya et al. 2020a). Pest causes up to 50% of potential crop loss, while 12.3% is supposed to be caused by nematodes and more damage to the crops due to nematode infestation is detected in the emerging countries than in the industrialized countries (Carneiro et al. 2017). Scientists have become interested in plant parasitic nematodes since they have been linked to significant, widespread diseases. There are many genera and species of plant parasitic nematodes that reduce the quality and quantity of yields in different crops while also raising the price of production. The crop of pigeon peas suffers significant damage from plant parasitic nematodes, which causes the plants' physiological processes to malfunction, lowering crop yield. The cyst nematode *H. cajani* has been shown to live on pigeon pea, and this parasite is by far the most prevalent (Briar et al. 2023). According to Ali and Askary (2001), *H. cajani* is among the most significant pests of pigeon pea in India, along with *M. incognita*, *M. javanica* and *R. reniformis*. According to Abd-Elgawad and Askary (2015), plant parasitic nematodes are responsible for a 13.2% yearly output loss in pigeon pea production globally. Adult females of *H. cajani* can be identified by their lemon-shaped forms, short necks and terminal cones. The cysts initially have a light yellow tint, but over time, they form strong walls, progress from yellow to brown to black, and eventually turn entirely black. *H. cajani* females are very different from females of other species. Their huge egg sac, which can occasionally be nearly twice as big as the cyst itself, is a distinguishing feature of these growths (Ali and Singh 2005). Edward and Misra (1968) reported *H. vigni* from the roots of pigeon pea (*Vigna unguiculata*), which was later discovered to be synonymous with *H. cajani*.

The use of *Trichoderma harzianum* as a biological control has been very effective in sustainable agricultural systems. The fungus is effective in controlling nematodes such as *Heterodera* (genera) by colonizing and trapping them (Tylka and Marett 2022), reducing disease through the predation of nematodes (Maina et al. 2022), its nematotoxic effects (Mhatre et al. 2022) and inhibiting egg hatching and juvenile mortality (Kumar et al. 2020). Therefore, the purpose of this investigation was to assess the effects of local *T. harzianum* strains found in Indian pigeon pea fields on the population of cysts, eggs and juveniles of *Heterodera cajani*.

Methods

Trichoderma harzianum—isolation, purification and identification

The process of isolation of *T. harzianum* was made from the rhizosphere of a healthy pigeon pea plant in the region of Prayagraj, UP in 10 different fields. The soil in the 10-cm rhizosphere was carefully scraped out from around the roots, which were then sliced into little pieces and mixed with the soil. *T. harzianum* were isolated using the serial dilution plate method (Sharma et al. 2019). The conidium morphology of *T. harzianum* can be used to quickly identify them. This species can be distinguished from all other *T. harzianum* by its small, subglobose conidia, which are typically 2–3 to 1.5–2.5 m in size. Conidiophores have a pyramidal, verticillate branching structure and lack a sterile apical elongation at maturity, despite the fact that developing conidiophores may show a visible sterile extension in the early stages of growth.

Collection, processed and identification of cyst nematode from infested soil

Infected soil was collected from different fields and processed by sieve methods. In this processing, 500 g of infested soil was passed through 25 mesh and then 60 mesh, the cyst of *Heterodera* (Da Silva Café and Santos 2023) *cajani* cannot pass through 60 mesh thus were collected carefully. The nematode suspension was stirred and poured in a small quantity in the counting dish. The counting dish was rotated under the low-power binocular microscope in search of cyst nematode. When the cyst appeared into the field, they were identified. The cysts were counted by using the counter and collected into the cavity block with the help of forceps (Maurya et al. 2022b) (Fig. 1). The female cyst is lemon shaped. The cuticle is marked by thick, zigzag lines. One large and one smaller annule is present on the head region (Koshy 1967). Cowpea seeds were sown for the mass multiplication of *H. cajani* in pot (Fig. 2).

Experimental site

Pots experiment was conducted at the playhouse of Sam Higginbottom University of Science and Technology, Prayagraj (UP), India, during the year of 2018–2020. Prayagraj was 98 masl and was sited at 25.27 levels north and 81.50 ranged east. Commonly, the climate is subtropical and semiarid. In the summer season, the maximum temperature might also get as excessive as 46.5 °C, and in the winter, it can reach up to 1.5 °C. Approximately, 1100 mm of rain precipitation takes place annually on common.

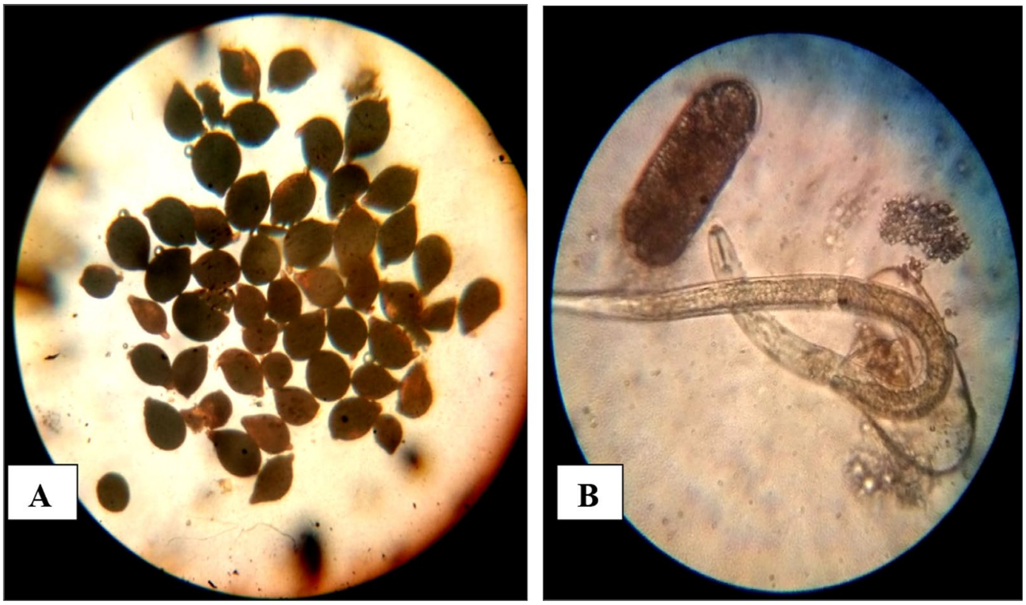

Fig. 1 Morphological identification of cyst nematode (*Heterodera cajani*), **A** Females of cyst nematode; **B** Hatching juveniles from egg of cyst

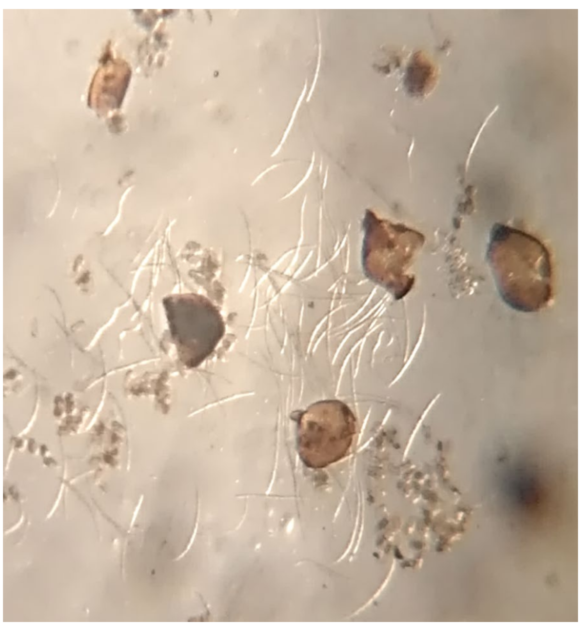

Fig. 2 Mass multiplication of *Heterodera cajani*

Preparation of treatments

The earthen pots were used for the experiment. The experiment's pots were washed in running water. The soil was collected and sterilized with 1% formalin solutions, earthen pots were filled with sterilized sandy loam soil @ 10 kg/pot for each replicate of the treatments. Before sowing the seed, about 5000 spores of *T. harzianum* (calculated by hemocytometer) were amended into the 10 kg of soil for proper colonization. Ten days after amendments of *T. harzianum,* seeds of pigeon pea (Upaas variety) were sown in each pot. In control pots, soil without amendment and one chemical (Carbofuran) were maintained for comparison. Fifteen days after seed germination, 200 cysts/ pot were inoculated near the root zone of pigeon pea plant. The pots were irrigated when required. Observations were recorded of cyst population, eggs and second juveniles of *H. cajani* (Min et al. 2020)/500gm of pigeon pea rhizospheric soil at 90 days after sowing.

Data analysis

A completely randomized design (CRD) was employed as the experimental design with three replications. A design of freedom (df) that was appropriate was created using the calculated value of F at a 5% level of probability (Chandel 2010; Abd-Elgawad 2021). Data expressed as percentages were transformed according to the angular transformation.

Results

First year (2018–2019)

The evaluation of *T. harzianum* on cyst, eggs and juveniles population in pigeon pea root and rhizospheric (500 g) soil was carried out. The result (Table 1 and Fig. 3) of the year 2018–2019 indicated that, at 90 days, *T. harzianum* isolates notably reduced the number of cyst population in the roots of pigeon pea plants as compared to control (nematode alone) (58). The maximum reduction of cyst population was recorded in T_{11}

Table 1 Evaluation the isolates of *Trichoderma harzianum* on cyst population, eggs and juveniles of *Heterodera cajani*/500 g of pigeon pea rhizospheric soil (2018–2019)

Treatments	Population (adults) at 90 DAS	No. of eggs/female cyst	No. of juveniles population/cyst
T_0-Control 200cyst	58	300	186
T_1-*T. harzianum* 1 + *H. cajani*	17	242	161
T_2-*T. harzianum* 2 + *H. cajani*	34	264	158
T_3-*T. harzianum* 3 + *H. cajani*	37	243	155
T_4-*T. harzianum* 4 + *H. cajani*	29	245	174
T_5-*T. harzianum* 5 + *H. cajani*	31	292	169
T_6-*T. harzianum* 6 + *H. cajani*	21	292	167
T_7-*T. harzianum* 7 + *H. cajani*	12	234	153
T_8-*T. harzianum* 8 + *H. cajani*	25	270	161
T_9-*T. harzianum* 9 + *H. cajani*	20	252	166
T_{10}-*T. harzianum* 10 + *H. cajani*	22	249	165
T_{11}-Carbofuran + *H. cajani*	6	197	135
F test	S	S	S
S.Ed (±)	0.69	0.90	0.48
C.D. ($P = 0.05$)	1.43	1.87	1.01

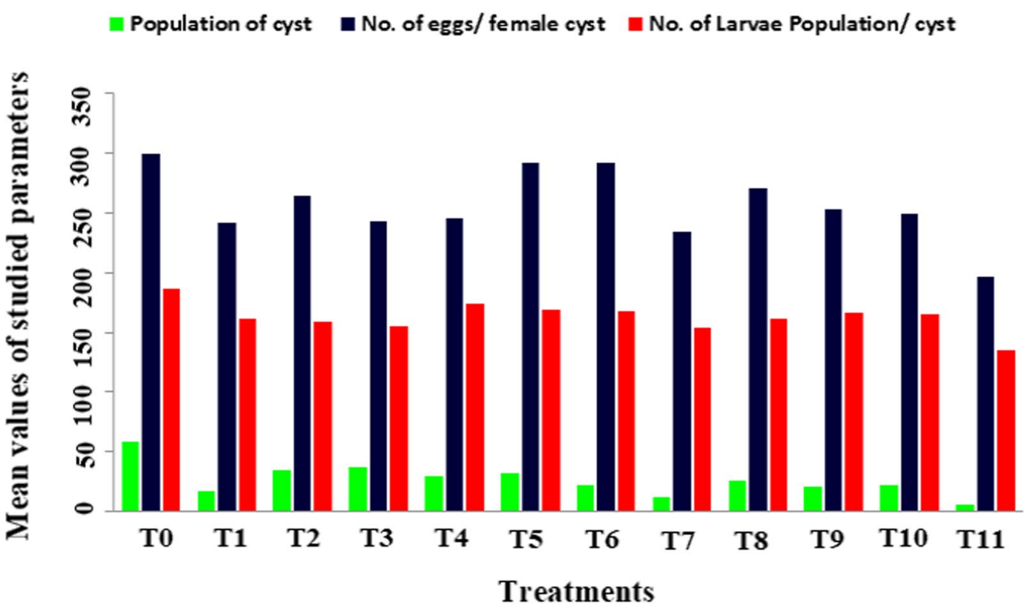

Fig. 3 Evaluation the isolates of *Trichoderma harzianum* on cyst population, eggs and juveniles of *Heterodera cajani*/500 g of pigeon pea rhizospheric soil (2018–2019)

(Carbofuran 6), T_7 (12) and T_1 (17), followed by T_9 (20), T_6 (21), T_{10} (22). The minimum reduction of cyst population was recorded in T_3 (37) and T_2 (34), followed by T5 (31), T4 (29) and T8 (25). The maximum reduction of egg population per cyst nematode female was also found in T_{11} (Carbofuran 197), T_7 (234), followed by T_1 (242), T_3 (243), T_4 (245). The minimum reduction of egg population was recorded in T_6 (292) and T_5 (292), followed by T_8 (270), T2 (264), T9 (252) and T10 (249).

The juveniles population/female cyst was also counted and it was observed that all isolates of the *T. harzianum* notably reduced the juveniles population of *H. cajani* in T_{11} (Carbofuran 135), followed by T_7 (153) T_3 (155), T_2 (158), T_1 (161) and T_8 (161). The minimum reduction of juveniles population in T_4 (179), followed by T5 (169), T6 (167), T9 (166) and T10 (165).

Table 2 Evaluation the isolates of *Trichoderma harzianum* on cyst population, eggs juveniles of *Heterodera cajani*/500 g of pigeon pea rhizospheric soil (2019–2020)

Treatments	Population of cyst at 90 DAS	No. of eggs/ female cyst	No. of juveniles population
T_0-Control 200cyst	65	309	201
T_1-*T. harzianum* 1 + *H. cajani*	14	235	183
T_2-*T. harzianum* 2 + *H. cajani*	32	255	147
T_3-*T. harzianum* 3 + *H. cajani*	36	235	146
T_4-*T. harzianum* 4 + *H. cajani*	27	239	170
T_5-*T. harzianum* 5 + *H. cajani*	29	290	164
T_6-*T. harzianum* 6 + *H. cajani*	20	284	160
T_7-*T. harzianum* 7 + *H. cajani*	11	189	160
T_8-*T. harzianum* 8 + *H. cajani*	21	259	144
T_9-*T. harzianum* 9 + *H. cajani*	19	243	158
T_{10}-*T. harzianum* 10 + *H. cajani*	21	241	153
T_{11}-Carbofuran + *H. cajani*	7	193	133
F test	S	S	S
S.Ed (±)	0.76	0.67	0.52
C.D. (P = 0.05)	1.57	1.40	1.08

Second year (2019–2020)

The result (Table 2 and Fig. 4) of the year 2019–2020 indicated that at 90th day, all the isolates of *T. harzianum* significantly reduced the number of cyst population in the roots of pigeon pea plants as compared to control (nematode alone) (65). The maximum reduction of cyst population was recorded in T_{11} (Carbofuran 7), T_7 (11) and T_1 (14), followed by T_9 (19), T_6 (20), T_8 (21) and T_{10} (21). The minimum reduction of cyst population was recorded in T_3 (36) and T_2 (32), followed by T_5 (29) and T_4 (27).

Each isolate of *T. harzianum* effectively reduced the eggs population of *H. cajani* than the control (309). The maximum reduction of egg population per cyst nematode female was also found in T_7-(T 189) and T_{11} (Carbofuran 193), followed by T_1 (235), T_3 (T 235), T_4 (T 239), T_{10} (241) and T_9 (243). The minimum reduction of egg population was recorded in T_5 (290) and T_6 (284), followed by T_8 (259) and T_2 (255).

Each isolate of *T. harzianum* suppressed the number of juveniles' population of *H. cajani* than the control (201). The maximum number of juveniles population /female cyst was counted and observed that all isolates of *T. harzianum* significantly suppressed the juveniles colony of *H. cajani* in T_{11} (Carbofuran 133), T_8 (144), T_3 (146), T_2 (147), followed by T_{10} (153) and T_9 (158). The minimum reduction of juveniles population in T_1 (183) and T_4 (170), followed by T_5 (164), T_7 (160) and T_6 (160).

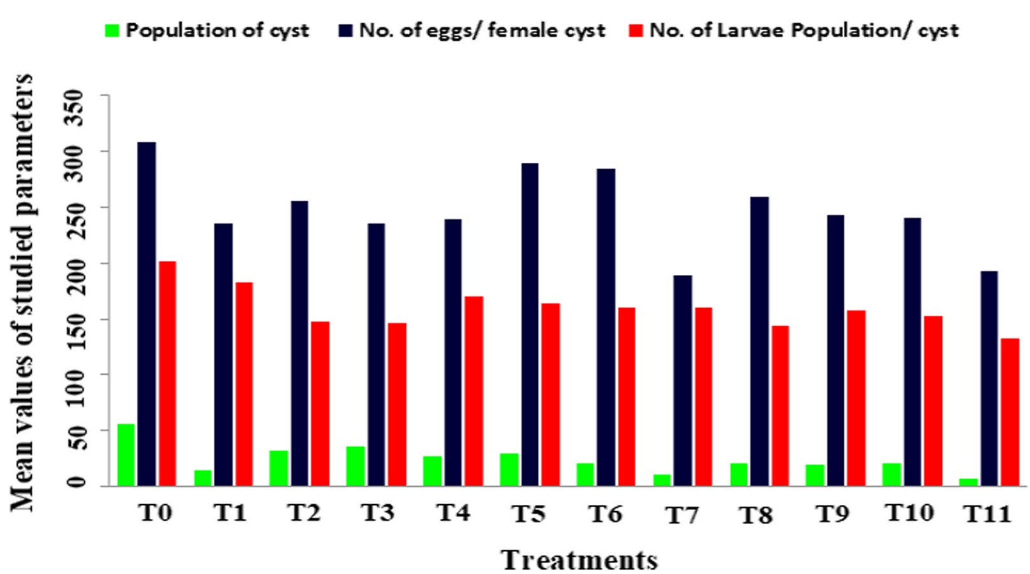

Fig. 4 Evaluation the isolates of *Trichoderma harzianum* on cyst population, eggs and juveniles of *Heterodera cajani*/500 g of pigeon pea rhizospheric soil (2019–2020)

Discussion

Conidiophores of *T. harzianum* have a pyramidal, verticillate branching structure and lack a sterile apical elongation at maturity, despite the fact that developing conidiophores may have a visible sterile extension in the early stages of growth. Ten distinct isolates of *T. harzianum* were collected and kept. The biocontrol agents of *Pochonia chlamydosporia* recorded 60.96 cyst populations as compared to 69.3 by *T. harzianum,* which was reported by Sinha et al. (2018). Similar work was performed by Sangma et al. (2022) where the antagonistic potential of *T. harzianum* was estimated in contradiction of *Heterodera cajani* of *Cajanus cajan* for 3 years. The solicitation of *T. harzianum* at 5 kg/ha significantly reduced the cyst nematode population (52.73) (Kalita 2020). Sinha et al. (2018) reported that maximum reduction in cyst nematode (*H. cajani*) multiplication was found in pigeon pea when *G. mosseae, T. harzianum* and *P. chlamydosporia* were used together. Use of *T. viride* reduced by the incidence of *H. cajani* on green gram, red gram and black gram (Alase et al. 2020). According to Carneiro et al. (2017), *Trichoderma* spp. had also been shown to colonize *Globodera rostochiensis* eggs and juveniles and devour the egg contents (Krif et al. 2020). *Trichoderma* spp. mineralize phosphorus in soil containing fungi (Guzmán-Guzmán et al. 2023). Sikora et al. (2021) stated that enzymes or other chemicals produced by fungi prevent the development of *Meloidogyne* and *Heterodera* in the rhizosphere in cooperation with the plant's root system. In order to offer significant information regarding these bioagents' effectiveness in controlling *G. rostochiensis* in the field, more research is required (Singh 2022). The bioagents *Fusarium* spp., *Trichoderma* spp., *B. thuringiensis* and *Saccharopolyspora spinosa* may cause a drop in nematode levels at the rates utilized in this experiment and may serve as a replacement control alternative to suppress *Meloidogyne* sp., *G. rostochiensis. G. rostochiensis'* growth was significantly inhibited by *Trichoderma* species. Reproduction rates dropped by 36.0–44.4% for both fungi and bacterial preparations by 27.7–33.3% than the controls (Mhatre et al. 2022). The findings of this study are very relevant since seven isolates of native *T. harzianum* are promising candidates to suppress the number of cysts, eggs and juveniles population of the phytopathogen nematode *H. cajani,* in addition to promote plant growth.

Conclusion

Seven isolates of the fungus *T. harzianum* are promising candidates to reduce the population of the nematode *H. cajani's* juveniles by 17.74 and 20.39%, eggs by 22.00 and 38.83%, and cysts by 79.31 and 83.07%. The level of 5000T. *harzianum* spores in 10 kg of soil had the capacity to reduce the number of *H. cajani* cysts, eggs and juveniles. The pigeon pea plant can grow more quickly thanks to *T. harzianum*. These local *Trichoderma* isolates can be regularly employed to control plant pathogenic nematodes that harm legumes.

Abbreviations

%	Percent
/	Per
@	At the rate of
°C	Degree centigrade
ANOVA	Analysis of variance
C f u	Colony forming units
cm	Centimeter
CD	Critical difference
DAS	Days after sowing
d.f.	Degree of freedom
ESS	Error sum of square
et al.	And others
etc.	And the rest
F (tab.)	Tabulated value of F
F (cal.)	Calculated value of F
gm	Gram
hrs.	Hours
i.e.	That is
MSS	Mean sum of square
TSS	Total sum of square
ml.	Milliliter
spp.	Species
viz.	Namely
T. s.	*Trichoderma* Spp
F.o.c.	*Fusarium udum*
H. c.	*Heterodera cajani*
CRD	Completely randomized design
T	Treatment

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Significant statement

This study is particularly beneficial to nematologists and plant pathologists since it offers significant details about the control of *Heterodera cajani* by *Trichoderma harzianum* on pigeon pea (*Cajanus cajan* L.) grown in a playhouse system.

Author contributions

The experiments were run and the paper was written by AKM. The information was evaluated and processed by VJ, DKS, NA and FB. The work was organized by HP, RS, AB, and AE, MYA. The manuscript was looked at and modified by HK, SHQ and ATQ. For final submission. The work was read and approved by all authors.

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Availability of data and materials

All data are available at the end of the article and the materials used in this work are of high quality and grade.

Declarations

Ethics approval and consent to participate

There are no researches conducted on animals or humans.

Consent for publication
The agreement of publication was taken.

Competing interests
The authors declare that they have no competing interests.

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