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Decoding *Pratylenchus thornei*: Reproductive Insights in Carrot Disc Culture and Bioagent Efficacy for Precision Chickpea Nematode Management

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ABSTRACT: This research delves into the reproductive behavior of *Pratylenchus thornei* in carrot disc culture, explores its vertical distribution in soil, and evaluates the effectiveness of various bioagents in mitigating nematode impact in chickpea cultivation. In carrot disc culture, the study reveals a maximum reproductive fitness of 19.56 fold after 40 days of inoculation, accompanied by a final nematode population of 489, comprising 84 females, 244 juveniles, and 161 eggs. Contrastingly, a minimum nematode population of 94 was observed after 20 days, with a reproductive fitness of 3.76. Extending the incubation period from 35 to 40 days yielded the highest reproductive rate, a pattern consistent with studies on *P. neglectus* and *P. zeae* in carrot disc cultures. Evaluation of bioagents for nematode management demonstrated their overall effectiveness, with *Purpureocillium lilacinum* exhibiting maximum efficacy. Utilizing this bioagent as seed treatment in JG 62 chickpea variety resulted in a final nematode population of 18.65 per 500 cc soil, 7.13 nematodes per gram of root, and a maximum plant height of 11.3. Similar efficacy was reported for *T. harzianum* and *P. lilacinum* in other studies, potentially attributed to the activation of defense genes. In conclusion, this study not only provides insights into the reproductive dynamics and vertical distribution of P. thornei but also underscores the promising potential of bioagents, particularly *Purpureocillium lilacinum*, for effective nematode management in chickpea cultivation.

Keywords: *Pratylenchus thornei*, Carrot disc culture, Bioagents, Nematode management, *Purpureocillium lilacinum.*

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is a crucial food legume with a global presence across 55 countries, encompassing an estimated 14.56 million hectares and yielding 14.78 million tons. Notably, India stands out as the leading producer, contributing 67.4% to the global production. Chickpea, rich in dietary protein and essential amino acids, plays a vital role in global food security, serving as a nitrogen fixer, contributing to soil fertility, and acting as a disease break. Despite its nutritional significance, chickpea faces challenges in achieving its potential yield of 6 t/ha, primarily due to biotic and abiotic stresses The world average chickpea yield is less than 1 t/ha which is far less than the potential yield of 6 t/ha under favorable and irrigated conditions (Varshney *et al*., 2017). The escalating productivity of chickpea since 1961 has also led to increased sensitivity to various stresses, resulting from the reuse of a limited number of germplasm accessions. Among biotic stresses, plant-parasitic nematodes, including *Pratylenchus thornei*, pose a significant threat, causing an estimated 14% loss in chickpea productivity. *Pratylenchus thornei*, a migratory endoparasite, penetrates, feeds, and migrates within the root cortex, leading to necrotic lesions and cavities, ultimately causing stunted growth and yield losses exceeding 50% in chickpeas. Globally distributed, *Pratylenchus thornei* has been identified as a major constraint to chickpea production in regions such as Turkey, where it affects 82% of chickpea fields. In Australia, this nematode causes yield losses of up to 40% in cereals and legumes. The challenges posed by *Pratylenchus thornei* necessitate effective management strategies.The root lesion nematodes, *Pratylenchus* spp. [*Pratylenchus thornei* Sher & Allen, 1953 (Tylenchida, Pratylenchidae)], is the most important constraint to legume production and have a wide distribution in many regions in Turkey (82% of chickpea fields) and affect many agricultural crops around the world (Tanha Maafi *et al*., 2009; Behmand *et al*., 2019).

Crop rotation with non-host crops, fallow periods, and the use of resistant cultivars are integral to integrated pest management. However, the broad host range of some nematode species limits the feasibility of crop rotations. Biological control agents, such as *Purpureocillium lilacinum* and *Pochonia chlamydosporia*, play a pivotal role in nematode

management. *Trichoderma* spp. is a globally identified successful bioagent which not only control plant diseases (Kumar *et al*., 2013a; Kumar *et al*., 2014; Jain *et al*., 2017; Kharte *et al*., 2022) but can also be used as biofertilizer (Srivastava *et al*., 2009) and in production of several secondary metabolites (Kumar *et al*., 2009). Accurate diagnosis, effective crop rotations or fallow periods, and tolerant/ resistant crop cultivars are three major elements for effective integrated control of plant-parasitic nematodes in chickpea (Thompson *et al*., 1995; Trudgill 1992). They are also helpful in plant growth promotion (Kumar and Sahu 2014; Kumar *et al*., 2019) and bioremediation (Kumar *et al*., 2015). Further, their use as a native isolate have proven better potential in the local area for successful bio-control agent after proper identification and characterization (Kumar *et al*., 2013b; Kumar and Sahu 2015; Kumar *et al*., 2016). Oyster mushroom (*Pleurotus ostreatus*) holds a potential to control the plant parasitic nematode both in-vitro and in-vivo (Kanaujiya *et al*., 2023). These agents demonstrate promising efficacy in reducing nematode populations, offering sustainable alternatives to chemical interventions (Ownley *et al*., 2004; Manzanilla-López *et al*., 2013). Achieving sustainable global food security will be a challenging task with the growing human population and shifting global food consumption patterns brought on by climate change (Kumar *et al*., 2021).

MATERIAL AND METHODS

A. Reproductive fitness of P. thornei populations in carrot disc culture

Carrot disc culture was used to identify the reproductive fitness of *P. thornei***.** Twenty five gravid females were transferred with a sterile handling needle into a drop of sterile distilled water on the carrot disc placed in sterilized Petri dishes. Inoculated discs in Petri dishes were incubated at 25.6°C with 16 and 8 hours of light and dark period for up to 40 days. The final nematode population (eggs, juveniles, and females) per carrot disc of *P. thornei* was estimated from carrot discs after 20, 25, 30, and 40 days after inoculation. After each incubation period, nematodes were extracted from carrot discs by centrifugation (Coolen, 1979). The protocol used for carrot disc culture inoculation is based on the work of Coyne *et al*. (2007).

The process of selecting and sterilizing *Pratylenchus thornei* (RLN) for inoculating carrot discs involved meticulous steps. Initially, 25 RLN females were carefully picked using a bamboo splinter and placed in a glass block with sterile distilled water. Streptomycin (6000 ppm) was added, and the solution was filtered through a 0.2 µm microfilter to minimize contamination. After settling, the nematode suspension underwent surface sterilization three times, alternating with streptomycin and sterile distilled water. Inoculation of carrot discs followed, with surface-sterilized nematodes gently placed on the discs in a laminar air flow. Petri dishes containing inoculated carrots were sealed and incubated at 25°C for up to 40 days, monitoring the development of callus as an indicator of healthy cultures. Harvesting was initiated when nematodes emerged onto the carrot surface. This method ensures controlled RLN inoculation in carrot disc cultures, contributing to comprehensive studies on nematode behavior and host interaction.

B. Evaluation of bio-agents against Pratylenchus thornei as seed treatment

The experiment was conducted under greenhouse condition. The soil composite filled earthern pots were infested with initial population of 1000 lesion nematodes. The talc based formulations of bio-agents obtained from IPL private limited, Haryana, were used during the course of investigations. Seeds of chickpea (JG 36) were treated with the talc formulation of bio-agent @ 10g/kg seeds (2×10^8spores/g talc). The pots were sown with treated/untreated seeds as per the treatments and irrigated. The experiment was allowed to run for 40 days and observations were recorded for final nematode population (soil/500 cm^3 and per 5g root) and plant height was taken. The population of *P. thornei* was assessed following the method described earlier. The experiment was laid on 12 Jan. 2021 and terminated on 26 Feb. 2021. The experiment was designed in Randomized Block Design (RBD) with seven treatments.

Treatment no.	Treatment detail	Dose
T_1	*Trichoderma harzianum*	10 g/kg seed
T_2	*Pochonia chlamydosporia*	
T_3	*Paecilomyces lilacinus*	
T_4	Ectomcorrhiza spp	
T_5	*Metarhizium anisopliae*	
T_6	*Beauveria bassiana*	
T_7	Control	-

RESULT

A. Reproductive fitness of P. thornei populations in carrot disc culture

Carrot disc culture was prepared to identify the reproductive fitness of *P. thornei* with an initial inoculums level of 25 nematodes per disc. Carrot inoculation and callus formation is presented in plate 5. There were significant differences in reproduction rate after four incubations of 20, 30, 35 and 40 days after inoculation. After 40 days of inoculation, maximum reproductive fitness of 19.56 fold was recorded with respect to initial population and the final nematode population of 489, consisting of 84 females 244 juveniles and 161 eggs were observed. This was followed by, a total population of 352 recorded after 35 days of inoculation with 59 females, 178 juveniles and 115 eggs and their reproductive fitness was 14.0. However, after 20 days of inoculation, minimum nematode population of 94 consisting of 18 females, 63 juveniles and 13 eggs could be recorded with a reproductive fitness of 3.76. After 30 days of inoculation, a population of 203 was recorded, consisting of 32 females, 109 juveniles and 62 eggs and reproductive fitness of 8.12 could be observed.

The experiment showed the gradual increase of reproductive fitness with increase in incubation period from 20 to 40 days. Maximum reproductive rate could be recorded on increasing the incubation period from 35 to 40 days. It was interesting to note that steep rise took

place in nematode population after 30 days onward and it showed quick increase in reproductive factor from 8.1 to 14.08 in 5 days. The detailed data for females, juveniles, and eggs of *P. thornei* with their reproductive fitness are given in Table 1 (Fig. 1A and 1B). The logarithmic regression analysis curve against the different incubation period is depicted in Fig. 1(C) with regression equation.

B. Evaluation of bio-agents against Pratylenchus thornei as seed treatment

Among the tested treatments for *Pratylenchus thornei* management in chickpea cultivation, *Purpureocillium lilacinum* (T3) demonstrated superior efficacy. With an initial nematode population of 100, *Purpureocillium lilacinum* achieved a final nematode population of 18.65 per 500 cm^3 soil and 7.13 nematodes per gram of root. The associated plant height was notably high at 11.83 cm, showcasing robust chickpea growth. This treatment out performed others, including *Trichoderma harzianum* (T1), which exhibited exceptional nematode control with a final nematode population of 59.11 per 500 cm3 soil and a plant height of 10.44 cm. *Pochonia chlamydosporium* (T2) displayed moderate efficacy, while Ectomycorrhiza (T4) showed relatively lower effectiveness. *Metarhizium anisopliae* (T5) and *Beauveria bassiana* (T6) demonstrated moderate control with positive effects on plant height. The control group (T7) exhibited the least effective nematode control, resulting in significantly higher final nematode populations and a lower plant height. The findings, supported by coefficients of variation (C.V.) and standard errors (SE), underscore *Purpureocillium lilacinum* as the most promising bioagent for sustainable *Pratylenchus thornei* management in chickpea cultivation. The detailed data for biocontrol agent and its impact on suppressing the *P. thornei* population and its effect on plant growth parameters is given in Table 2 (Fig. 2A and B). These results highlight the potential for improving chickpea yields and nematode resistance in agricultural settings.

DISCUSSION

In carrot disc culture, maximum reproductive fitness of 19.56fold was recorded after 40 days of inoculation, and the final nematode population of 489, consisting of 84 females 244 juveniles and 161 eggs could be observed. However, after 20 days, minimum nematode population of 94 was obtained with a reproductive fitness of 3.76. Maximum reproductive rate could be recorded on increasing the incubation period from 35 to 40 days. The similar findings were reported while working with carrot disc cultures of *P. neglectus* (Esteves *et al.*, 2015) and *P. zeae* (Kagoda *et al.*, 2010). The *P. neglectus* was able to reproduce in 56 days after inoculation (DAI) and the final population densities significantly increased at 84 DAI. The differences in multiplication period/rate may be attributed due to different species.

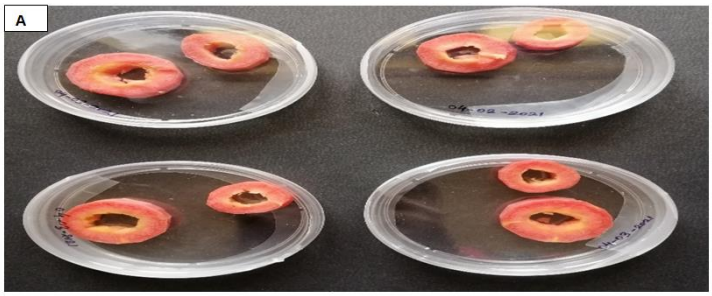
To identify the vertical distribution of *P. thornei*in soil, maximum population of 5314 RLN per 500 cc soil was present at the depth of 12 to 16 cm. However, the minimum RLN population of 635 per 500 cc soil was recorded at 0-4 cm of soil depth. Being the desi chickpea grown in the field and high temperature during harvesting and migratory nature of *P. thornei* may be attributed for presence of major population of 3460.12 per 500 cc soil of *P. thornei* upto the depth of 16-20 cm. Smiley *et al.*, (2008) also reported greater population of *P. thornei* in the upper surface upto 30 cm, while exploring 0 to 120 cm depth in Oregon.

Table 1: Effect of different incubation period on population of *P. thornei* in carrot disc culture.

Time (days)	Number of nematodes			Pf	Rf
	Female	Juveniles	Eggs		
20	18	63	13	94	3.76
30	32	109	62	203	8.12
35	59	178	115	352	14.08
40	84	244	161	489	19.56

Table 2: Effect of biocontrol agents on the population of *Pratylenchus thornei*

Treatment	Treatment detail	Initial nematode population	Final nematode population		Plant height (cm)
			soil/500 cm^3	Root/g	
T1	Seed treatment *with Trichoderma harzianum*@ 10g/kg seed	100	59.11 (1.77)	11.23 (1.05)	10.44
T2	Seed treatment with *Pochonia chlamydosporium*@ 10g/kg seed	100	49.78 (1.70)	8.43 (0.92)	9.57
T3	Seed treatment with *Purpureocillium lilacinum*@ 10g/kg seed	100	18.65 (1.27)	7.13 (0.85)	11.83
T4	Seed treatment with *Ectomycorrhiza* @ 10g/kg seed	100	154 (2.18)	26.2 (1.41)	8.74
T5	Seed treatment with *Metarhizium anisopliae* @ 10g/kg seed	100	21.87 (1.33)	9.34 (0.97)	9.57
T6	Seed treatment with *Beauveria bassiana*@ 10g/kg seed	100	26 (1.41)	12.46 (1.09)	9.52
T7	Control	100	277.28 (2.44)	36.18 (1.55)	7.86
	C.D.		**5.839**	**0.314**	**0.188**
	SE(m)		**1.972**	**0.106**	**0.064**
	SE(d)		**2.789**	**0.15**	**0.09**
	C.V.		**4.551**	**1.339**	**1.301**



A. Carrot discs treated with ethanol and stored in BOD.

B. Carrot disc treated with nematodes (after callus formation).

Plate- 1

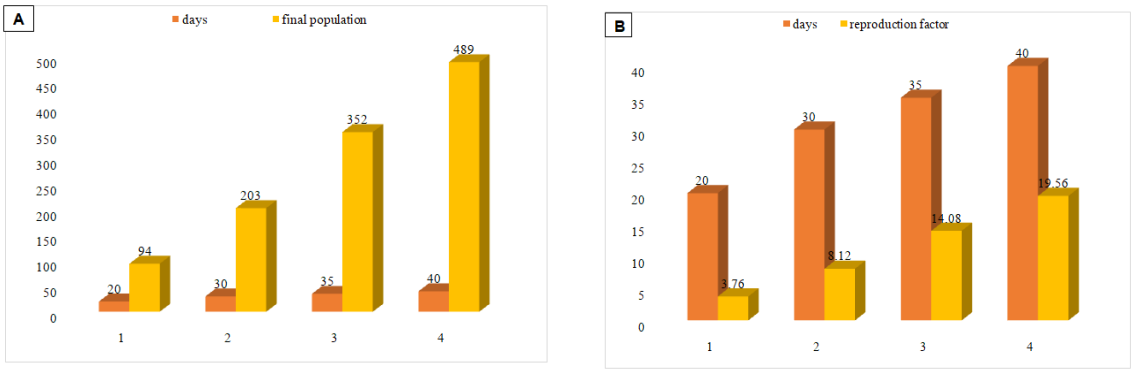


Fig. 1(A) Final nematode population after different days. **Fig. 1(B)** Reproductive factor after different days.

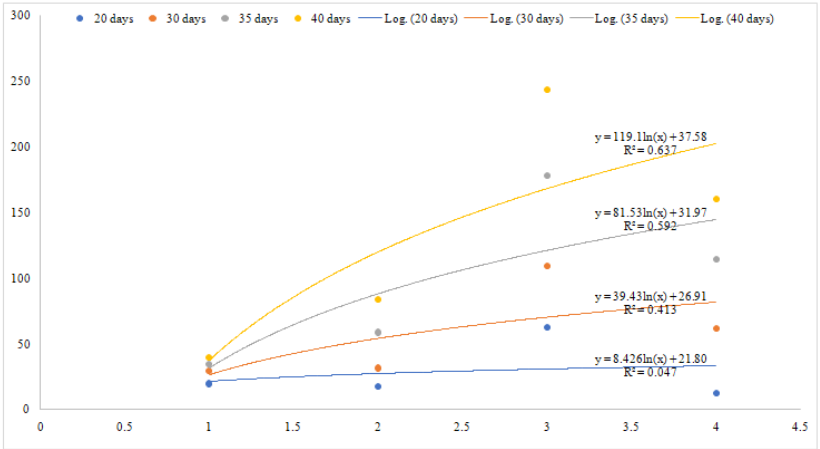


Fig. 1(C). Logarithmic regression analysis curve against the different incubation period.

All the evaluated bioagents found effective in mitigating the effect of *P. thornei* in soil. However, *Purpureocillium lilacinum* exhibited maximum efficacy and the final nematode population was 18.65 per 500cc soil and 7.13 nematodes per gram of root with maximum plant height of 11.3 using seed treatment in JG 62 chickpea variety. Similarly, efficacy of *T. harzianum*@5g/Kg seed, *Purpureocillium lilacinum* @ 10^8 conidial culture (Kepenekci*et al.*, 2018) have been reported against *P. thornei* in different studies in chickpea. This may be attributed due to activation of defense genes as reported by Tolba *et al.*, 2021 while working on root endophytism using *Pochonia chlamydosporia* which affected defense-gene expression in leaves of monocot and dicot hosts under multiple biotic interactions. The gene expression patterns in leaves exhibited specific and time-dependent relationship between host plants and *P. chlamydosporia*

in presence of biotic stress factors like *Pratylenchus goodeyi*.

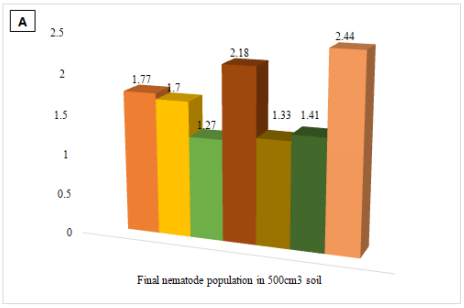


Fig. 2(A). Final nematode population in 500cm^3 soil.

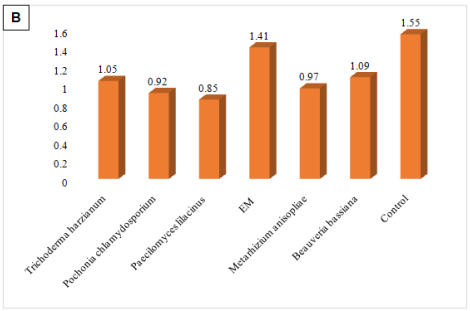


Fig. 2(B). Final nematode population in Root/gm.

CONCLUSIONS

In this study, the reproductive dynamics of *Pratylenchus thornei* in carrot disc culture, its vertical distribution in soil, and the efficacy of bioagents in chickpea nematode management were thoroughly investigated. Carrot disc culture experiments revealed significant variations in reproductive fitness over different incubation periods, with a maximum fold increase of 19.56 observed after 40 days. The vertical distribution of *P. thornei* in soil showed a peak population at 12-16 cm depth, emphasizing the impact of chickpea cultivation practices on nematode presence. Evaluation of bioagents demonstrated their effectiveness, with *Purpureocillium lilacinum* exhibiting maximum efficacy in reducing nematode populations in soil and roots.

FUTURE SCOPE

For future research directions, I suggest exploring species-specific variations in the reproductive dynamics of *Pratylenchus thornei*, building on observed distinctions with *P. neglectus* and *P. zeae*. Extending our findings to encompass long-term field studies would provide valuable insights into the practical implications of nematode behavior in real-world crop cultivation settings. Investigating the molecular and physiological mechanisms that underlie the efficacy of bioagents, especially *Purpureocillium lilacinum*, is crucial for a more comprehensive understanding of their mode of action against P. thornei. Additionally, researchers should consider exploring synergies by integrating bioagents with existing nematode control strategies, aiming for sustainable chickpea cultivation practices. Further investigation into the intricate interactions between *Pochonia chlamydosporia* and host plants, particularly regarding defense-gene expression under various biotic stress conditions, would contribute significantly to advancing our knowledge in this field. By addressing these avenues, future research can contribute to more effective nematode management strategies and enhance the sustainability of chickpea cultivation.

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Conflict of Interest. None.

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