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Effect of *Purpureocillium lilacinum* on Root Lesion Nematode, *Pratylenchus thornei*

İlker KEPENEKCİ[a], Halil TOKTAY[b], Erçin OKSAL[c], Refik BOZBUĞA[d], Mustafa İMREN[e]

[a]*Gaziosmanpasa University, Faculty of Agriculture, Department of Plant Protection, Tokat, TURKEY*
[b]*Nigde Omer Halisdemir University, Faculty of Agricultural Sciences and Technologies, Department of Plant Production and Technologies, Nigde, TURKEY*
[c]*Inonu University, Faculty of Agriculture, Department of Plant Protection, Malatya, TURKEY*
[d]*Biological Control Research Institute, Nematology Laboratory, Adana, TURKEY*
[e]*Abant Izzet Baysal University, Faculty of Agriculture and Natural Science, Department of Plant Protection, Bolu, TURKEY*

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Corresponding Author: Erçin OKSAL, E-mail: ercin.oksal@inonu.edu.tr, Tel: +90 (422) 377 30 00
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ABSTRACT

Wheat (*Triticum* spp.) is highly susceptible to the root lesion nematode, *Pratylenchus thornei*, which causes excessive amounts of crop losses each year. In this research, we investigated the cumulative effect of the biocontrol agent *Purpureocillium lilacinum* (syn: *Paecilomyces lilacinus*) against *P. thornei*. Three doses of *P. lilacinum* (10^6, 10^7 and 10^8 conidia cultures mL^{-1}) with one dose of 400 *P. thornei* individuals (adults and juveniles) mL^{-1} were applied in 100 cm^3 soil under greenhouse conditions. The number of nematodes in the soil and root in addition to total nematode in soil+root were determined. Moreover, different plant parameters such as the plant height, plant fresh and dry weight, root fresh and dry weight were evaluated. Applications with the higher dose of bio-agents (100 cm^3 *P. thornei* infested soil with 10^8 conidia culture of *P. lilacinum* mL^{-1}) exhibited maximal enhancement in dry and fresh weight of shoot and reduced *P. thornei* population. As a consequence, *P. lilacinum* individually was highly effective in enhancing different plant parameters and suppressing *P. thornei* reproduction. Overall, present findings suggest that the exploitation of the biocontrol agent *P. lilacinum* could be helpful for effective management of the root lesion nematode *P. thornei*.

Keywords: *Pratylenchus thornei*; *Purpureocillium lilacinum*; Wheat; Biological control

1. Introduction

Wheat (*Triticum* spp.) is the most important crop in the world. It is cultivated in many countries and the second most important food crop in the developing world following rice. However, despite its high value, the wheat is highly vulnerable against to different types of viral, bacterial, fungal, and nematode pathogens, and insect pests. Nematodes are very important among these pests affecting wheat production. The root lesion nematodes (RLN), *Pratylenchus* species, is the most dominant group among the plant parasitic nematodes. It is ubiquitous in almost all cereal fields and causes vast crop losses every year (Nicol 2002).

RLN are widely distributed and have wide host ranges (Nicol 2002). Host suitability and abiotic factors such as soil type and moisture level chiefly govern the distribution and determine yield losses caused of RLN (Orion et al 1984). RLN compromise ninety-seven valid species distributed in almost every environmental condition in the world (Handoo et al 2008). Recently, Smiley (2010) reported that at least eight species of RLN could infect cereal crops. Among them, *P. thornei* and *P. neglectus* were reported as two very devastating species.

As a cereal root eelworm, *P. thornei* (Sher and Allen) (Tylenchida: Pratylenchidae) has been reported to feed and reproduce inside wheat roots, causing considerable loss of plant vigor and reduction in grain yield (Thompson & Clewett 1986). It is a migratory polyphagous endoparasitic nematode that causes necrotic lesions on the root systems of host crops. It is reported to be a pathogen of wheat throughout the world, with the exception of South America (Nicol 2002). *P. thornei* causes yield losses of wheat of 85%, 70%, 50% and 37% in Australia, Israel, America and Turkey, respectively (Toktay 2008; Smiley 2010; Imren et al 2015).

Several chemical nematicides have been recommended to reduce the reproduction of these nematodes. Particularly, the nematicides used to control *Pratylenchus* species have been considered less effective, environmental pollutant, toxic to the beneficial soil microflora, and expensive methods (Thomason 1987; Carneiro et al 2007).

For successful biological control, whether indigenous or introduced, the presence of antagonistic organisms in the environment is prerequisite. To date, numerous antagonistic organisms have been reported capable of reducing populations of plant parasitic nematodes (Stirling 2011). As a common soil inhabitant, various bacteria (i.e. *Pasteuria* spp.) and fungi (i.e. *Purpureocillium lilacinum*, *Pochonia chlamydosporia*) have been reported parasitizing nematodes in soil (Stirling 1991).

Purpureocillium lilacinum (Thom) Luangsaard, Hywel-Jones, Houbraken and Samson (syn: *Paecilomyces lilacinus*) (Sordariomycetes: Hypocreales) is a typical soil-borne fungus (Luangsa-Ard et al 2011). It was isolated firstly from insects in tropical regions. It has been recorded in many regions of the world specifically in warm regions (Domsch et al 1980). *P. lilacinum* was determined to cause infection in many insect, nematode and acarian species. Also, it has been described to be as efficient as commonly used nematicides (Dube & Smart 1987; Mendoza et al 2007). It is the most important egg and female parasite of plant parasitic nematodes (Morgan-Jones et al 1984; Dube & Smart 1987; Atkins et al 2005; Khan et al 2006). Furthermore, there are a lot of isolates of *P. lilacinus* and many commercial products especially the "strain 251" of *P. lilacinus* is being used as a biological control agent against nematodes all over the world (Atkins et al 2005).

The main objective of this study was to evaluate the efficacy of the fungal parasite, *P. lilacinum* and determine its effective dose of application for management of *P. thornei* on wheat in vitro conditions. This study is a pioneer research to investigate the pathogenicity of indigenous *P. lilacinum* against *P. thornei* in Turkey.

2. Material and Methods

2.1. Preparation of nematode inoculum

The root lesion nematodes, *P. thornei* which sourced from the Biological Control Research Station in Adana, Turkey, were cultured in vitro on carrot discs as described by Moody et al (1973). The nematode suspensions were prepared with sterile water. After suspending in a 50 mL of tap water in a beaker, *P. thornei* individuals (adults and juveniles) were counted using a binocular microscope. One mL of water consisting of 400 individuals was then injected to each plant a week after sowing.

2.2. Preparation of fungal source

The entomopathogenic fungus, *P. lilacinum* TR1 was provided from the Plant Protection Central Research Institute, Ankara, Turkey. *P. lilacinum* TR1 was determined (Kepenekci et al 2009) and identified by classical and molecular methods (Kepenekci et al 2015). *P. lilacinum* was isolated

from root-knot nematodes in the tomato plant roots in Sarıcakaya province (Eskişehir, Turkey). Potato Dextrose Agar (PDA) medium was used to raise a fresh culture of *P. lilacinum*. The wheat grains were inoculated with conidia scrapped from two weeks old plates. The grains were mixed following an incubation period of three weeks at 25±1 °C. To obtain a uniform suspension of fungal conidia, 500 mg of culture was mixed in 30 mL of 0.05% sterile agar solution. The number of conidia per mL was calculated by hemocytometer. The serial dilutions were prepared and the number of conidia was measured under hemocytometer to achieve the 10^6, 10^7 and 10^8 conidia mL^{-1} concentrations.

2.3. Experimental set-up

The study was carried out in 6 cm diameter pots containing 100 cm^3 of sterilized sandy loam soil (ca. 70% sand, 29% clay, and 1% organic matter). Wheat variety of Seri-82 seeds were transplanted into pots after germination on a moistened filter paper in Petri dishes. Three doses of *P. lilacinum* i.e. 10^6, 10^7 and 10^8 conidia cultures mL^{-1} with one dose of 400 *P. thornei* individuals (adults and juveniles) mL^{-1} were tested either alone or in combination. Also, the positive control (control +) *P. thornei*, and the negative control (control -) only water were added. Moreover, the number of nematodes into soil and root along with the total nematode in soil+root were counted in each treatment. Furthermore, different plant parameters such as the plant height, plant fresh and dry weight and also root fresh and dry weight were evaluated.

Each treatment was tested in a randomized complete block design with seven replicates. Plants were watered as needed and no fertilizer was added. Plants were grown under controlled conditions at temperatures between 20-25 °C, 16 hours of artificial light, and 70% relative humidity from March to May in 2013. The nematodes were extracted using a Baermann funnel after 9 weeks of nematode inoculation (Hooper et al 2005) from soil and the roots of harvested plants. One milliliter of extracted nematode suspension was counted with three replications in a counting slide at 32-fold magnification under a stereomicroscope. The number of nematodes was calculated in per plant. Fresh shoots were measured and weighed then they were dried at 70 °C for 48 hours to determine the dry weight.

2.4. Statistical analysis

Data were analyzed according to standard analysis of variance procedures using SPSS 10.0 program for Windows (SPSS 1999). Differences among treatments were tested using one-way analysis of variance (ANOVA) followed by the Duncan Test for mean comparison, if the F-value was significant ($P<0.05$).

3. Results

The experiments evaluated the effect of *P. lilacinum* on the population of *P. thornei* based on the number of nematodes in soil and root and total nematode in soil+root. Applications of various *P. lilacinum* reversed the adverse effect of nematode multiplication. Among the different doses of *P. lilacinum* conidia cultures, the maximum effect on the nematodes was recorded using the treatment having applications of higher doses of the bio-agent (Table 1).

Table 1- Effect of *Purpureocillium lilacinum* on number of *Pratylenchus thornei

P. lilacinum treatments (conidia mL^{-1})	*Number of nematodes in soil (pot containing 100 cm^3 soil)*	*Number of nematodes in root (pot containing one plant root)*	*Number of total nematode in soil+root*
10^6	3502±924.2 b	2360±25.1 c	5862±915.6 c
10^7	2458±365.1 b	2480±38.4 c	4938±262.2 bc
10^8	2192±331.5 b	1484±59.2 b	3676±353.3 b
Only *P. thornei*	5456±358.0 c	3128±117.3 d	8584±442.7 d
Only water	0 a	0 a	0 a

*, means in the same column followed by a different letter are significantly different according to LSD test ($P\leq0.05$)

Moreover, the numbers of nematodes in the soil were not significantly different based on the different doses of *P. lilacinum*. However, the number of nematodes in the soil for the control treatment (-) was significantly different to those observed for the various treatments of *P. lilacinum*. The best effects on the reducing the multiplication of nematode in the root were observed using the highest concentration of *P. lilacinum*. Additionally, the minimum nematode values were observed for the greatest concentration of *P. lilacinum* in the soil and root (Table 1).

The experiments assessed the effect of *P. lilacinum* and *P. thornei* on plant height, plant fresh and dry weight. In general, different doses of *P. lilacinum* caused positive effects on plant growth. Among the various treatments evaluated, maximal increases in the dry weight were recorded in the treatment having applications of higher doses of the bio-agents. The control (+) treatment using only *P. thornei* also had similar results to these higher *P. lilacinum* dose treatments. Conversely, the applications of *P. lilacinum* at three dosages were not found to have any significant effect on the improvement of shoot weight and plant height (Table 2).

Furthermore, the plant height and the upper parts of plant (shoot) fresh were not significantly different based on the different doses of *P. lilacinum*. Conversely, control (-) treatment resulted in significant differences in these two plant parameters. Alternatively, the dry weights (g) of plant were significantly different with the higher dose of *P. lilacinum* (Table 2).

Table 2- Effect of *Purpureocillium lilacinum* on growth characters of plants infected by *Pratylenchus thornei**

P. lilacinum treatments (conidia mL^{-1})	*Plant height (cm)*	*Fresh weight of plant (g)*	*Dry weight of plant (g)*
10^6	26.8±2.27 b	0.32±0.03 b	0.15±0.02 c
10^7	27.2±6.85 b	0.44±0.08 b	0.22±0.02 c
10^8	38.8±3.37 b	0.63±0.09 b	0.40±0.10 b
Only *P. thornei*	28.0±2.81 b	0.49±0.07 b	0.42±0.06 b
Only water	56.0±1.38 a	1.46±0.23 a	0.81±0.05 a

*, means in the same column followed by a different letter are significantly different according to LSD test (P≤0.05)

The higher dose of the bioagent was also observed to cause maximal improvement in the root fresh weight. With regard to root weight, a similar trend was also visible. Higher doses of *P. lilacinum* reduced the adverse effect of nematodes on the dry root weight. Generally, improvements in fresh and dry weight of root were produced by the higher dose of *P. lilacinum* (Table 3).

As a result, the best effects highest concentration of *P. lilacinum* on the multiplication of nematode were determined 2192, 1484 and 3676 *P. thornei* individuals (adults and juveniles) soil, root and soil-root respectively. Also, the best treatment using higher doses of *P. lilacinum* yielded maximal improvement in fresh weight of shoot and root and increase in dry weight of shoot and root respectively.

Table 3- Effect of *Purpureocillium lilacinum* on various growth characters of roots infected by *Pratylenchus thornei**

P. lilacinum treatments (conidia mL^{-1})	*Root fresh weight (g)*	*Root dry weight (g)*
10^6	0.57±0.05 b	0.17±0.03 b
10^7	0.47±0.13 b	0.34±0.14 b
10^8	1.20±0.17 a	0.78±0.06 a
Only *P. thornei*	0.44±0.08 b	0.19±0.05 b
Only water	1.45±0.11 a	0.71±0.09 a

*, means in the same column followed by a different letter are significantly different according to LSD test (P≤0.05)

4. Discussion

The biological control agent, *P. lilacinum* individually was highly effective in enhancing different plant parameters such as plant growth, fresh and dry weight of shoot and suppressing *P. thornei* population in the present study. Also, amongst the various treatments tested, applications with the higher dose of bio-agent (i.e. 100 cm^3 *P. thornei* infested soil with 10^8 conidia culture of *P. lilacinum* mL^{-1}) showed maximal improvement in fresh and dry weight of shoot and root.

Our results are in conformity with that of Rao & Malek (1973), who observed nematode-trapping fungi such as *Arthrobotrys dactyloides*, *A. arthrobotryoides* and *Dactylaria thaumesia* slowed the population increase of *P. penetrans* on alfalfa and determined that among the fungi tested, *A. dactyloides* was the most effective antagonist. Similarly, Mai et al (1977) reported different the nematode-trapping fungi such as *Arthrobotrys superba*, *A. dactyloides*, *A. arthrobotryoides* and *Dactylella doedycoides* reduced penetration of alfalfa roots by *P. penetrans*. Khan et al (2006) who observed that entomopathogenic fungi *Hirsutella rhossiliensis* suppresses the root lesion species, *P. penetrans* and *P. neglectus.* Moreover, Di Zahao et al (2013) reported that entomopathogenic fungi *P. lilacinus* impact the multiplication of different nematodes such as cyst nematodes *Globodera pallida* and *Heterodera* spp; root knot nematodes, *Meloidogyne incognita*.

Altogether, findings in the present study suggest that the exploitation of bio-control agent *P. lilacinum* could be helpful for effective management of the root lesion nematode. Based on the observations of this study it could be concluded that *P. lilacinum* had a cumulative effect on reduction of nematode population and improvement of plant growth. There are variations based on the different conidia doses of *P. lilacinum* and this difference may be the result of the variation in the experimental conditions or might be due to the environmental effects on the performance of bio-agent. It is also important in that the pathogenicity of *P. lilacinum* on *P. thornei* has not been studied under greenhouse conditions before this study. Although the result of this study gave a promising outcome, additional comprehensive research is needed to ascertain the capacity of nematode antagonists to suppress populations of *P. thornei in vivo* conditions.

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