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Original article (Orijinal araştırma)

Investigation of the development of root lesion nematodes, *Pratylenchus* spp. (Tylenchida: Pratylenchidae) in three chickpea cultivars

Kök lezyon nematodlarının, *Pratylenchus* spp. (Tylenchida: Pratylenchidae) üç nohut çeşidinde gelişmesinin incelenmesi

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Abstract

In this study, penetration, population changes and reproduction rates of root lesion nematodes, *Pratylenchus neglectus* (Rensch, 1924), *Pratylenchus penetrans* (Cobb, 1917) and *Pratylenchus thornei* Sher & Allen, 1953 (Tylenchida: Pratylenchidae), at 3, 7, 14, 21, 28, 35, 42, 49 and 56 d after inoculation in chickpea Bari 2, Bari 3 (*Cicer reticulatum* Ladiz) and Cermi [*Cicer echinospermum* P.H.Davis (Fabales: Fabaceae)] were assessed in a controlled environment room in 2018-2019. No juveniles were observed in the roots in the first 3 d after inoculation. Although, population density of *P. thornei* reached the highest in Cermi (21 d), Bari 3 (42 d) and the lowest observed on Bari 2. *Pratylenchus neglectus* reached the highest population density in Bari 3 and Cermi on day 28. The population density of *P. neglectus* was the lowest in Bari 2. Also, population density of *P. penetrans* reached the highest in Bari 3 cultivar within 49 d, similar to *P. thornei*, whereas Bari 2 and Cermi had low population densities during the entire experimental period.

Keywords: Chickpea, penetration, population density, *Pratylenchus*, reproduction

Öz

Bu çalışmada, Bari 2, Bari 3 (*Cicer reticulatum* Ladiz) ve Cermi [*Cicer echinospermum* P.H.Davis (Fabales: Fabaceae)] nohut çeşitlerinde Kök lezyon nematodlarının, *Pratylenchus neglectus* (Rensch, 1924), *Pratylenchus penetrans* (Cobb, 1917) ve *Pratylenchus thornei* Sher & Allen, 1953 (Tylenchida: Pratylenchidae), 3, 7, 14, 21, 28, 35, 42, 49 ve 56 günlerde popülasyon değişimleri, penetrasyon ve üreme oranları kontrollü oda koşullarında 2018-2019 yılları arasında incelenmiştir. İlk 3 günde nohut çeşitlerinin köklerinde larvalar görülmemiştir. *Pratylenchus thornei* en yüksek popülasyon yoğunluğuna Cermi çeşidinde 21 günde, Bari 3 çeşidinde 42 günde ulaşmasına rağmen Bari 2 çeşidinde üreme en düşük düzeyde kalmıştır. *P. neglectus* Bari 3 ve Cermi çeşidinde 28 günde en yüksek popülasyon yoğunluğuna ulaşmıştır. Bari 2'de ise en düşük popülasyon yoğunluğu *P. neglectus*'da görülmüştür. Buna ek olarak, *P. penetrans* popülasyon yoğunluğu, *P. thornei*'ye benzer şekilde, 49 günde Bari 3 çeşidinde en yüksek seviyeye ulaşırken, Bari 2 ve Cermi çeşitlerinde deneme süresince düşük popülasyon yoğunluğu göstermiştir.

Anahtar sözcükler: Nohut, penetrasyon, popülasyon yoğunluğu, *Pratylenchus*, üreme

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Introduction

Chickpea [*Cicer arietinum* L. (Fabales: Fabaceae)] is one of the most important food legumes in the world. It is an ancient crop that has been grown in India, the Middle East and parts of Africa for many years. It may have been grown in Turkey in the twelfth century BC. (Singh & Ocampo, 1997). The chickpea growing area in Turkey is about 514 kha with a production of 630 kt and an average yield of 1.2 t/ha (TUIK, 2018). Chickpea production in Turkey has increased over recent decades. Turkey is ranked fifth in the world for chickpea production (FAO, 2019). It has been reported that plant parasitic nematodes can cause 21-40% crop losses in chickpea crops (Ali & Sharma, 2003; Reen et al., 2014). The strategies for nematode management hinge on detection and population density estimation to keep the nematode population below an economic threshold. However, this is difficult to achieve and needs to be modified under different cultivation conditions (Abd-Elgawad & Askary, 2015). Chemical control is not economic for use in crop production on large areas. Therefore, the most effective control method is to consistently use of resistant cultivars (Roberts, 2002).

Taylor et al. (2000) reported that there are differences between crops and cultivars in host susceptibility to root lesion nematode, *Pratylenchus neglectus* (Rensch, 1924) (Tylenchida: Pratylenchidae). For example, chickpea, wheat and canola were good hosts whereas field pea, faba bean and triticale were poor hosts. Thirty-three plant parasitic nematode species have been identified in association with chickpeas in Turkey (Kepenekçi & Ökten, 2000). Root lesion nematodes are one of the most important plant parasitic nematodes that causing damage to chickpea crops in Turkey (Di Vito et al., 1994b; Kepenekçi, 1999) and elsewhere in the world (Sharma et al., 1992).

There have been a range of studies on root penetration and population dynamics of root lesion nematodes. Rebois & Huettel (1986) indicated that there was a significant difference between the number of nematodes in the roots and agar in soybean and corn; the population density of eggs and individuals in the roots was greatest than nematode population in agar. *Pratylenchus thornei* Sher & Allen, 1953 (Tylenchida: Pratylenchidae) was found on 24 plant species that reproduced well on 97 chickpea lines in Syria and Turkey (Greco et al., 1988; Gomez-Barcina et al., 1996; Behmand et al., 2019). There are different factors that can affected on root lesion [*P. thornei* and *Pratylenchus penetrans* (Cobb, 1917) (Tylenchida: Pratylenchidae)] egg hatching. Pudasaini et al. (2008) indicated that the temperature of 20ºC had a greater effect on egg hatching among these factors. Population dynamics of *P. thornei* were observed in the chickpea genotypes grown in the greenhouse for 16 weeks (Thompson et al., 2015). The root lesion nematodes including *P. neglectus, P. penetrans* and *P. thornei*, have been commonly determined in fields in Turkey (Şahin et al., 2008; Söğüt & Devran, 2011; Akyazi et al., 2018). Also, Behmand et al. (2019) showed that these nematodes have been found widely distributed in 82% of the chickpea fields in many regions in Turkey.

Information on population density, population dynamics and growth rate in root is very important in the control of root lesion nematodes and to help keep the nematode population density below an economic threshold. The aim of the study was to determine population dynamics of root lesion nematodes, *P. neglectus, P. penetrans* and *P. thornei,* as well as root penetration rates, and root-feeding behavior on resistant and susceptible chickpea cultivars under laboratory conditions.

Materials and Methods

Plant materials

Chickpea cultivars that were determined previously as resistant and susceptible to *P. neglectus, P. penetrans* and *P. thornei* by Behmand (2020) were screened against these root lesion nematodes (Table 1). These cultivars used in an experiment at Plant Protection Department, Çukurova University in 2018 -2019.

Table 1. Chickpea cultivars used for investigation of the population dynamics of *Pratylenchus neglectus*, *P. penetrans* and *P. thornei* in experiments

Chickpea species	Chickpea cultivars	Reaction*
Cicer echinospermum	Cermi 063	S
Cicer reticulatum	Bari3 106	S
Cicer reticulatum	Bari2 062	R

*R: Resistance S: Susceptible

The chickpea seed was surface sterilized and germinated in an incubator. The seeds were first washed with 30% ethanol for 1 min, rinsed in sterile water and placed in 4% sodium hypochlorite solution for 45 s, and then washed twice with sterile pure water. The seeds were sacrificed by making a small cut on the seed coat to improve water absorption and incubated at 21ºC for 5 d to germinate on moist filter paper in 90-mm Petri dishes. Seeds were sowed singly into sterilized tubes containing 60 g sterilized soil. The tubes were placed in a controlled environment room at 20-25ºC.

Nematode cultures

Nematodes used in this study were collected during April and May 2014 to 2016 from a chickpea field (37º08’29" N 38º46’30" E) in Harran District, Şanlıurfa p8province, Turkey (Behmand et al., 2019). The climate is arid to semiarid in that area. The average soil temperature at a depth of 20 cm was 9.4ºC from April to May and average of 460 mm of annual rainfall and average RH of about 49% during April and May in 2014 to 2016. Mass cultures of *P. neglectus, P. penetrans* and *P. thornei* were grown in carrot discs in Plant Protection Department, Çukurova University and used as inoculum for the experiments (Moody et al., 1973).

Experiment

The soil was autoclaved at 121ºC for 20 min to kill nematodes and other plant pathogens (Baker, 1962). Autoclaved tubes with a capacity of 60 g (diameter: 2.5 cm, height: 15 cm) were used to grow the plants. Germinated seeds were planted singularly in the tubes supported by a box frame in the controlled environment rooms according to the randomized split-plot design with four replicates. The main plots where the nematodes species, and subplots the wild *Cicer* species [*Cicer echinospermum* P.H.Davis and *Cicer reticulatum* Ladiz (Fabales: Fabaceae)].

One week after sowing, the seedlings were inoculated with number of 225 nematodes consisting of mixed development stages in 1 ml of water (Behmand et al., 2020).

Chickpea cultivars were harvested at 3, 7, 14, 21, 28, 35, 42, 49 and 56 d after planting and all roots from each tube processed. The roots in each tube were carefully washed away and then stained in acid fuchsine to visualize the nematodes (10 ml 1% acid fuchsine, 17.5 ml lactic acid, 12.6 ml glycerin, 12.4 ml pure water) (Moltmann, 1988). At each harvest, population density of nematodes was counted from all stained roots under microscope type Leica 4000B. Reproduction factor (RF) was calculated as Pf/Pi, (the ratio of the final and initial nematode population densities) (Keil et al., 2009). Optimization validation within this study showed that nematodes were entered chickpea roots mostly by day 7. Therefore, Pi values were used from the day 7 assessment.

Data analysis

The data at each assessment time were subject to analysis of variance (ANOVA) and means were compared Tukey multiple range test at the $P \leq 0.05$ significance level using SPSS statistical program (Version 25, SPSS, Inc, Chicago, IL, USA). Analyses were performed on nematode density data normalized by using the $\log_{10}(x + 1)$ transformation. Also, a repeated measure analysis of variance (ANOVA) was used to examine interactions of day and nematode species.

Results and Discussion

An experiment was conducted to study population development and reproduction rates of *P. neglectus, P. penetrans* and *P. thornei* on Bari 2, Bari 3 and Cermi chickpea roots in 56 d the controlled environment room. It was determined that *P. neglectus, P. penetrans* and *P. thornei* entered the roots of Bari 2, Bari 3 and Cermi at 3-7 d after nematode inoculation (Table 2). *Pratylenchus neglectus, P. penetrans* and *P. thornei* were observed feeding at several sites in the roots during all migratory stages of their life cycles.

The highest RF (Table 2) obtained was 44.6 for *P. thornei* in Cermi followed by RF of 36.6 for *P. neglectus* in Bari 3 and 25.4 for *P. penetrans* in Bari 3.

Table 2. Population densities and reproduction factors (RF) for *Pratylenchus neglectus*, *P. penetrans* and *P. thornei* in chickpea roots

Assessment day	Cultivar	*Pratylenchus thornei* (mean ± SE)[1]	RF	*Pratylenchus neglectus* (mean ± SE)	RF	*Pratylenchus penetrans* (mean ± SE)	RF
3	Bari3	0 ± 0	0	0 ± 0	0	0 ± 0	0
	Bari2	0 ± 0	0	0 ± 0	0	0 ± 0	0
	Cermi	0 ± 0	0	0 ± 0	0	0 ± 0	0
7	Bari3	11.2 ± 1.4 a	1	5.2 ± 1.2 b	1	6.2 ± 1.3 ab	1
	Bari2	7.5 ± 1.1 a	1	5.5 ± 1.0 b	1	2.7 ± 0.8 b	1
	Cermi	8 ± 1.8 a	1	13.2 ± 1.3 a	1	11.5 ± 1.3 a	1
14	Bari3	29.7 ± 5.6 a	2.6	32.7 ± 2.6 a	6.2	13 ± 0.7 ab	2.1
	Bari2	34.2 ± 9.9 a	4.6	10.7 ± 0.4 c	2	8.5 ± 1.5 b	3.1
	Cermi	56 ± 8.6 a	7	20.2 ± 0.8 b	1.5	19.7 ± 2.9 a	1.7
21	Bari3	15.5 ± 7.6 b	1.4	3.7 ± 0.7 b	0.7	7.5 ± 2.1 a	1.2
	Bari2	11.2 ± 6.3 b	1.5	21.7 ± 8.1 a	4	11.2 ± 1.9 a	4.1
	Cermi	356.5 ± 20.7 a	44.6	13.7 ± 1.6 a	1	8.7 ± 0.4 a	0.8
28	Bari3	27 ± 6.3 b	2.4	192.2 ± 66.5 a	36.6	64 ± 31.8 a	10.2
	Bari2	31.7 ± 5.7 b	4.2	18 ± 2.6 b	3.3	65.5 ± 46.4 a	23.8
	Cermi	73.5 ± 1.3 a	9.2	186 ± 79.9 a	14	52.2 ± 25.0 a	4.5
35	Bari3	25.2 ± 3.8 b	2.2	73.5 ± 24.7 a	14	71.5 ± 25.3 a	11.4
	Bari2	17.5 ± 1.0 b	2.3	11 ± 4.8 a	2	65.2 ± 17.4 a	23.7
	Cermi	69.7 ± 0.8 a	8.7	95 ± 43.0 a	7.2	37.7 ± 1.1a	3.3
42	Bari3	57.5 ± 5.5 a	5.1	25 ± 8.6 ab	4.8	94.7 ± 19.3 a	15.2
	Bari2	6.7 ± 1.6 b	0.9	16.7 ± 2.9 b	3	17.5 ± 2.2 b	6.4
	Cermi	78.7 ± 0.8 a	9.8	144.7 ± 48.7 a	10.9	80 ± 9.4 a	7
49	Bari3	11.2 ± 1.9 b	1	41.5 ± 6.5 a	7.9	158.5 ± 3.1 a	25.4
	Bari2	13.7 ± 1.4 b	1.8	16.5 ± 1.9 b	3	15 ± 2.0 c	5.5
	Cermi	100 ± 1.0 a	12.5	51 ± 5.2 a	3,8	45.2 ± 3.3 b	3.9
56	Bari3	30.2 ± 1.0 a	2.7	37.7 ± 2.7 b	7.2	53 ± 20.2 a	8.5
	Bari2	17.7 ± 1.1 a	2.4	18.7 ± 1.2 c	3.4	17 ± 0.9 a	6.2
	Cermi	50.2 ± 16.7 a	6.3	106.2 ± 3.1 a	8	65 ± 2.8 a	5.7

[1] Data are means of four replicates ± standard errors, and numbers within columns followed by the same letter are not significantly different (P < 0,05) according to Tukey multiple range test using transformed data, $\log_{10}(x + 1)$; in each harvest time between cultivars; RF (reproduction factor) = Pf (final population density) / Pi (initial population density).

Over the course of the experiment, the population density of nematodes generally peaked twice. The population density reached its first peak on days 21 to 28, depending on the species and cultivar. *Pratylenchus thornei* reached its highest population density on day 21, and *P. neglectus* and *P. penetrans* on day 28 (Table 2).

Population density of *P. thornei* peaked on day 21 and then again on day 49 in Cermi. It is concluded that *P. thornei* completed its first life cycle in 21 d (Figure 1). *Pratylenchus thornei* reached highest population density on 42 d in Bari 3 whereas low population development was observed in Bari 2. The RF of *P. thornei* in Cermi was higher than in Bari 2 and Bari 3 over the entire experiment (Figure 1). Also, the second highest population density for *P. thornei* was reached after 49 d and the RF decreased to 12.5 in Cermi (Table 2). Based on the population development of *P. thornei* in the roots of chickpea cultivars used in the experiment (Figure 1), it is suggested that Cermi is a susceptible cultivar and the Bari 2 and Bari 3 are resistant or tolerant cultivars.

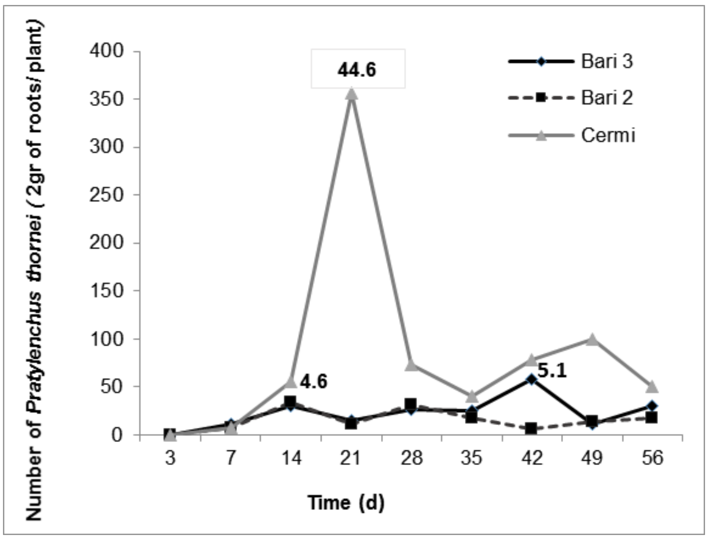

Figure 1. Population changes of *Pratylenchus thornei* in chickpea cultivars, Bari 2, Bari 3 and Cermi.

Pratylenchus neglectus reached the highest population density on day 28 in Bari 3 and Cermi but remained at a low density in Bari 2. *Pratylenchus neglectus* had three peaks in Cermi during the its development period whereas there was only one peak for Bari 3. The highest *P. neglectus* population density was on day 28. The RF for *P. neglectus* was 36.6 and 14.0 on day 28 in Bari 3 and Cermi, respectively. Based on the data shown in Figure 2, it is suggested that Bari 2 was the most resistant cultivar with RF of *P. neglectus* not exceeding 4 whereas Bari 3 and Cermi are susceptible to *P. neglectus*. Also, it is concluded that *P. neglectus* completed first life cycle in 28 d in Bari 3 and Cermi.

The population development of *P. penetrans* reached its first peak on day 28 in Bari 2, Bari 3 and Cermi (Figure 3). Also, the RF of *P. penetrans* in Bari 3 was higher than in Cermi and Bari 2 after 49 d whereas *P. neglectus* had two peaks in the three chickpea cultivars during the experiment. Consequently, it was found that the interaction of the day, day by nematode species, day cultivar, day by nematode species by cultivar were significant at $P < 0.0001$, whereas there was no significant difference observed between nematode species. Therefore, all nematode species and day factors were important in this study (Table 3).

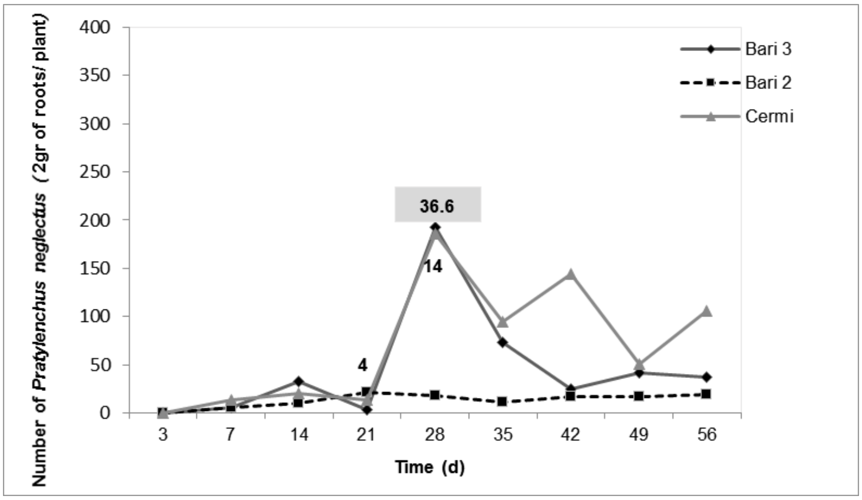

Figure 2. Population changes of *Pratylenchus neglectus* in chickpea cultivars, Bari 2, Bari 3 and Cermi.

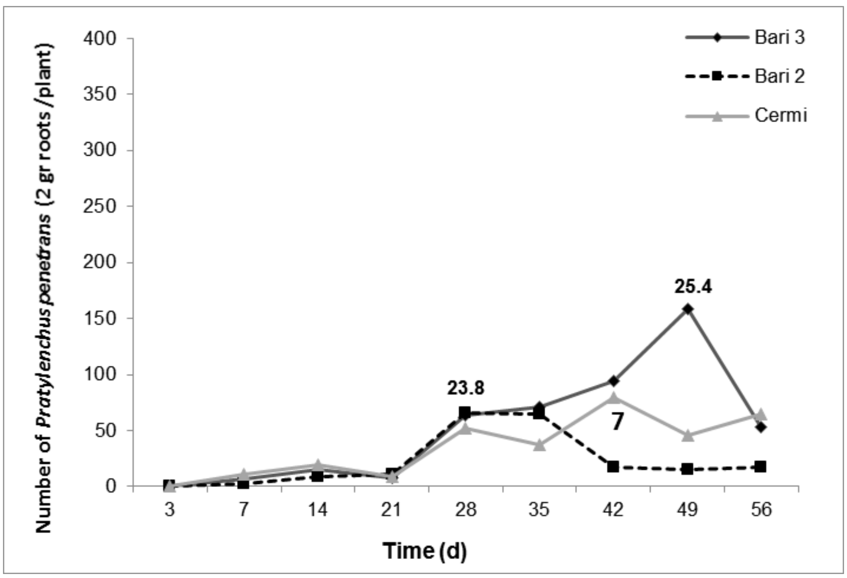

Figure 3. Population changes of *Pratylenchus penetrans* in chickpea cultivars, Bari 2, Bari 3 and Cermi.

Table 3. Variance analysis of the roots of chickpea cultivars (Bari 2, Bari 3 and Cermi) were infected with *Pratylenchus neglectus*, *P. penetrans* and *P. thornei* on different days

Source of Variation	df	MS	F	P
Day	8	22725.693	20.577	<0.0001
Day × nematode species	16	11318.867	10.248	<0.0001
Day × cultivar	16	6924.716	6.270	<0.0001
Day × nematode species × cultivar	32	8896.289	8.055	<0.0001
Error (day)	216	1104.447		
Intercept	1	535661.346	413.591	<0.0001
Nematode species	2	1271.123	0.981	>0.05
Cultivar	2	60787.077	46.935	<0.0001
Nematode species × cultivar	4	16769.660	12.948	<0.0001
Error	27	1295.146		

df, degree of freedom; MS, mean square; F and P values.

Chickpea is an important legume crop produced in most provinces of Turkey. Economic importance and damage of root lesion nematodes *i*on plants can be measured by the estimation of population density of nematodes per unit of root and/or soil, or by RF (Tiwari et al., 1992; Di Vito et al., 1995; Thompson et al., 2011; Reen et al., 2019). Ali & Ahmad (2000) demonstrated that the lesions present on infected roots are only symptoms and are not a direct measure of nematode numbers. Factors such as the population density of nematodes and the nematode life cycle have an important effect on the damage to the crop and assessing these will be useful for controlling of nematode population below an economic threshold.

Currently, there are only few studies that have investigated the development of root lesion nematodes on different chickpea cultivars in root and soil. High and low population densities were established to develop an evaluation of nematodes by growing susceptible and resistant chickpea cultivars (Taylor et al., 2000). At planting, the damaging threshold of root lesion nematodes can change the nematode life cycles in chickpea cultivars. This occurs because of the competition between nematode species and the different temperature requirements of the nematode in the soil or root. So, the population density of nematodes in soil may not provide useful information on the number of nematodes in roots. However, if root lesion nematodes are present in the soil then actively managing for root lesion nematodes should be considered. According to the results, there was a significant difference between nematode species in the root sample, and population density of nematodes differed between cultivars of *C. echinospermum* and *C. reticulatum* studied. Behmand et al. (2020) indicated that the population density of root lesion nematodes was differed with species (*P. neglectus* and *P. thornei*) and *Cicer* species. The population density of *P. thornei* in *C. echinospermum* was higher than the population density in *C. reticulatum*. A similar study by Thomson (2011) showed that the RF of root lesion nematodes differed between *C. echinospermum* and *C. reticulatum*, and RF of *P. thornei* in *C. reticulatum* was more than the RF in *C. echinospermum*. Also, the present study found that all root lesion nematodes penetrated roots after the inoculation by days 3 to 7. Peak populations of *P. neglectus, P. penetrans* and *P. thornei* were greatest in Cermi. These findings indicated that Cermi is more susceptible than Bari 2 and Bari 3 to *P. thornei*. Also, the life cycle was completed in different times for nematode species, for *P. thornei* the population density was highest on day 21, and the others on day 28. The development of the population density in to *P. thornei* changed between resistance and susceptible cultivars. A study by Linsell et al. (2014) indicated that the population density of *P. thornei* was reduced in resistance wheat cultivars and Vanstone et al. (2008) showed the full cycle from egg to adult is completed within 45 to 65 d and is greatly influenced by the host, temperature and *Pratylenchus* spp. Hatching activity may decrease with increasing plant age in different plant cultivar roots (Umesh & Ferris, 1992; Pudasaini et al., 2008).

The population density of *P. neglectus* in Bari 3 and Cermi was higher than in Bari 2 on days 28 and 35, however, the population density of *P. penetrans* in Bari 2 on days 42 and 49 was higher. The differences in penetration and population density of these nematodes in similar cultivars at the end of the 56-day experiments might be due plant physiological differences and nematode virulence in these cultivars. Penetration time of nematode species differs between crops. For example, nematodes penetrated *Brassica rapa* L. (Brassicaceae) within the first 6 h after inoculation whereas in *Zea mays* L. (Poaceae) penetration was within 8 to 12 h (Ogiga & Estey, 1975).

Crop loss can be associated with the population density of nematodes in the soil (Olthof & Potter, 1972). However, knowing the number of nematodes in the root is important in order to control root lesion nematodes. The population changes of *Pratylenchus neglectus, P. penetrans* and *P. thornei* were evaluated inside the roots of chickpea to help chickpea breeding programs.

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