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Original article (Orijinal araştırma)

Development of methodology for resistance screening of chickpea genotypes collected in Turkey to the root lesion nematode, *Pratylenchus thornei* Sher & Allen, 1953 (Tylenchida: Pratylenchidae)[1]

Türkiye'den toplanan nohut genotiplerinin kök yara nematoduna, *Pratylenchus thornei* Sher & Allen, 1953 (Tylenchida: Pratylenchidae) karşı taranması

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Abstract

The root lesion nematode, *Pratylenchus thornei* Sher & Allen, 1953 (Tylenchida: Pratylenchidae) are considered economically important plant parasitic nematodes affecting chickpea [*Cicer arietinum* L. (Fabales: Fabaceae)] production. A major strategy to develop resistance to root lesion nematodes in chickpea is to assess and exploit their natural variation. Therefore, nine accessions of wild *Cicer reticulatum* Ladiz. (Fabales: Fabaceae), *Cicer echinospermum* P.H.Davis (Fabales: Fabaceae) and domesticate *C. arietinum* were assessed for resistance to *P. thornei* according to multiplication rate of the nematode. This study was conducted during 2014-2015 to detect the suitable initial inoculum density (150, 225 and 300) per plant duration of experiment (16 and 20 weeks) for resistance test to *P. thornei* in chickpea cultivars. There was no significant difference between growing times 16 and 20 weeks and between the initial inoculum density of 225 and 300 nematodes. The only significant difference was observed at a low initial inoculum density of 150 nematodes in all tested cultivars. Therefore, the initial inoculum density of 225 and the growing time of 16 weeks were selected to access of chickpea genotypes for resistance study to *P. thornei*.

Keywords: Chickpea, optimization, *Pratylenchus thornei*, resistance, screening method

Öz

Kök yara nematodu, *Pratylenchus thornei* Sher & Allen, 1953 (Tylenchida: Pratylenchidae) dünyada nohut [*Cicer arietinum* L. (Fabales: Fabaceae)] üretimini etkileyen, ekonomik açıdan önemli bitki paraziti nematod türü olarak kabul edilmektedir. Nohutta kök yara nematodlarına karşı direnç geliştirmenin temel stratejisi, doğal çeşitliliklerini değerlendirmek ve kullanmaktır. Bundan dolayı *P. thornei*'ye karşı dayanıklılık çalışmalarında, üreme oranı dikkate alınarak, dokuz adet yabani *Cicer reticulatum* Ladiz. (Fabales: Fabaceae), *Cicer echinospermum* P.H.Davis (Fabales: Fabaceae) ve yerli *C. arietinum* türleri değerlendirilmiştir. Bu çalışma, nohut çeşitlerinin *P. thornei*'ye karşı dayanıklılık denemeleri için, uygun hasat süresi (16 ve 20 hafta) ve bitki başına başlangıç inokulum yoğunluğunu (150, 225 ve 300) belirlemek için 2014-2015 yılları arasında yürütülmüştür. Hasat zamanı 16 ve 20 hafta ile 225 ve 300 başlangıç inokulum yoğunluğu arasında anlamlı bir fark olmadığı, buna karşın bitki başına 150 inokulum yoğunluğunun istatistiki olarak daha düşük etki gösterdiği saptanmıştır. Bu nedenle, nohut çeşitlerinde *P. thornei*'ye karşı dayanıklılık çalışmaları için en uygun deneme parametrelerinin, 225 başlangıç inokulum yoğunluğu ve 16 hafta hasat zamanı olduğu belirlenmiştir.

Anahtar sözcükler: Nohut, optimizasyon, *Pratylenchus thornei,* dayanıklılık, tarama yöntemi

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Introduction

Chickpea [*Cicer arietinum* L. (Fabales: Fabaceae)] has an important place in total legume production in the world. The most important chickpea producing countries are India, Australia, Myanmar, Ethiopia, Turkey, Pakistan, Russia, Iran, Mexico, USA, and Canada (FAO, 2019). Turkey is ranked fifth in the world for chickpea production (FAO, 2019). It may have been grown and widely cultivated as a food legume in Turkey 7500 years ago (Singh & Ocampo, 1997). More species of plant parasitic nematodes have been found in the root and rhizosphere of the chickpea production areas in the world. Plant parasitic nematodes generally feed on different parts of the plant, especially on roots and other subterranean plant structures such as rhizomes in legume crops. Sasser & Freckman (1987) reported that yield losses in chickpea caused by several plant parasitic nematode species was about 13.7% worldwide. Also, Sikora et al. (2005) showed nematode damage to chickpea can make plant sensitive to disease and other stress. Similarly, Atkinson et al. (1995) showed that they are major pests of agriculture crops and have global economic effects on crops of more than 100 billion USD every year by the way. The root lesion nematodes, *Pratylenchus* spp. [*Pratylenchus thornei* Sher & Allen, 1953 and *Pratylenchus neglectus* Rensch, 1924 (Tylenchida, Pratylenchidae)], are the most important constraint to legume production and have a wide distribution in 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). Root lesion nematodes in chickpea need to be surveyed periodically and conducted risk analysis, based on its economic importance.

Pratylenchus thornei, P. neglectus, *Pratylenchus penetrans* Cobb, 1917 and *Pratylenchus crenatus* Loof, 1960 (Tylenchida: Pratylenchidae), are the most important root lesion nematodes in the world (Vanstone et al., 1998). Among the root lesion nematodes, *P. thornei* and *P. neglectus* are globally distributed and they enter the root tissue of host plant for feeding and reproduction (Nicol et al., 2004). Also, some studies indicated that in the terms of damage caused by these nematodes is second importance as a nematode problem in the world after root-knot nematodes (Barker & Noe, 1987; Jatala & Bridge, 1990). *Pratylenchus thornei* is one of the most important plant parasitic nematodes causing yield losses of up to 40% in cereals and legumes in dryland cropping areas of southeastern Australia (Thompson et al., 1995; Vanstone, 1998). These nematodes have been found widely distributed in a wheat fields in Turkey (Behmand et al., 2019). However, study of resistance screening methods for chickpea is limited in Turkey. Standardization of factors is important for development of a screening methodology that is stable and able to distinguish various levels of resistance. Thompson et al. (2010) reported that among the root lesion nematodes, *P. thornei* was a major problem in the Australian grain area. Similarly, Taylor et al. (2000) showed that both *P. thornei* and *P. neglectus* are important in chickpea in Australia. Chickpea, *C. arietinum* infested with root lesion nematodes showed symptoms of stunted growth and leaf chlorosis. *Pratylenchus* spp. infection causes symptoms of reduction in root hairs or nodules, and causes yield losses greater than 50% in chickpeas (Castilo & Jimenez, 1998; Castilo & Vovlas, 2007). Thompson et al. (2000) reported that an integrated pest management strategy including rotation with non-host crops or fallow period, and use of resistant cultivar is the best method to control nematode population in the cropping system involving grain legumes and cereals. Similarly, Trudgill (1992) indicated that the use of tolerant cultivars can grow and yield well in the extremely infested region with plant parasitic nematodes and can keep the population density below damage threshold levels.

Population density of nematodes is influenced by the reproduction potential and various factors such as initial population density and growing period. Identification of factors such as initial population density of nematodes and harvest time to keep the nematode population below damage threshold levels is very important in a screening study. Estimating threshold levels and calculating economic thresholds for most nematode/crop problems is not yet possible. Consequently, information on the relationship between initial population densities of nematodes and crop performance is necessary to obtain such data. An

understanding of the information in these relationships is basic to being able to predict yield reductions from estimates of pre-planting nematode population densities (Pi).

There is little diversity of resistance genes in *C. arietinum* cultivars to plant parasitic nematodes (Smýkal et al., 2015). New collections of *Cicer reticulatum* Ladiz. and *Cicer echinospermum* P.H.Davis (Fabales: Fabaceae) have been found to have high genetic diversity compared to domestic chickpea (Thompson et al., 2011). A similar study by Thompson et al. (2000) with a limited number of the accession of wild (*C. reticulatum* and *C. echinospermum*) and domesticated *C. arietinum* showed that the wild *Cicer* species can grow and have better tolerance in soil heavily infested with nematodes, and can be used in chickpea breeding programs for nematode resistance.

The aim of the study was to establish a reliable methodology for assessing wild and domesticated chickpea accessions for resistance to *P. thornei* by using different initial inoculation density and time of assessment.

Materials and Methods

Chickpea accessions

In this study, the accessions evaluated included three landraces (Eğil, Kalkan and Şırnak) of *C. reticulatum*, three landraces (Destek, Karabahçe and Ortance) of *C. echinospermum* and three cultivars (Azkan, Çağatay and Gökçe) of *C. arietinum* collected from Diyarbakır, Şanlıurfa and Şırnak Provinces of Turkey by the Department of Field Crops, Harran University. These were assessed in the laboratory for resistance to *P. thornei* under controlled conditions.

The seeds were scarified by making a small cut in the seed coat before germinating to improve water absorption and germination in the wild genotypes. The individual chickpea seeds were disinfected with hypochlorite (4%) and alcohol (30%) pre-germination and placed on the surface of wet filter paper in sterile Petri dishes and seeds were germinated at 21°C for 3 d.

Nematode source

Pratylenchus thornei was cultured on carrot discs by using the method described of Nicol and Vanstone (1993). Nematodes used this study were originally collected from a chickpea production area in Şanlıurfa Province (Harran District) located in the Southeastern Anatolia Region of Turkey and reproduced in the nematology laboratory at Çukurova University. A total of root and soil samples were collected from chickpea field between June and July 2014. Each root and soil samples included 5-10 samples (taken at a depth of 10-20 cm), with a total of 1-2 kg of soil/sample. Nematodes were extracted from both of the roots and soil with using the Baermann funnel technique in the laboratory (Hooper, 1986).

Experimental design

The study was conducted in completely randomized block with four replicates. One germinated seed each was sown into small open-ended tubes (16 cm high, 2.5 cm diameter) that contained of 60 g field soil, (73% clay, 16.5% silt and 10% river sand) that had been autoclaved for 2 h at 121°C.

One week after planting, the nematodes were transferred to room temperature at 25°C and plants were inoculated with either 150, 225 or 300 nematodes/tube in 1 ml water.

Experiment was conducted at the same time with all of the plant accessions and grown in a growth room at 25°C and 50% RH under a 16:8 h L:D photoperiod provided by high pressure sodium lamps.

Assessment of nematode multiplication

After 16 and 20 weeks, plant shoots were removed and the nematodes extracted from roots and soils by the Baermann funnel technique (Hooper, 1986). Then 1 mL of a suspension including nematodes was counted with four replicates in a counting slide under a light microscope and the total number of the nematodes extracted from plant and soil calculated. Multiplication rate (MR) of *P. thornei* was calculated MR = Pf/Pi, where Pf is the final and Pi is the initial nematode population density. For this purpose, the initial and final populations were the number of nematodes/tube.

Statistical analysis

The number of nematodes/tube from the experimental plots were analyzed using a completely randomized design ANOVA in Genstat (V13). Significant differences among treatments and replication of data were calculated at $P < 0.001$. Outliers and variance distribution were assessed using residual plots.

Results

Effect of harvest time on nematode multiplication

There was no significant difference between testing period time (16 and 20 weeks) and *Cicer* species $P > 0.001$ (Table 1). It means the multiplication rate of *P. thornei* was not differentiated among chickpea cultivars when the time of harvest changed from 16 to 20 weeks. However, the development of population density of *P. thornei* in *C. arietinum* was higher than the population density of *C. reticulatum* and *C. echinospermum*. Also, the population density of *P. thornei* in *C. echinospermum* except for 16 weeks when inoculated with 300 nematodes as Pi was lower than the population density of *C. reticulatum* and *C. arietinum* in both growing times of 16 and 20 weeks under laboratory condition (Figure 1).

Effect of initial Inoculum density on nematode multiplication

There was a significant difference between the initial inoculation densities of 150, 225 and 300 nematodes/tube ($P < 0.001$). The nematode density had a major effect on final numbers ($P < 0 .001$) and there is some indication of an interaction between chickpea species and the linear effect of nematode density ($P < 0.001$) (Table 1). Figure 2 shows the final population density of *P. thornei* increasing at initial inoculum densities of 150-225 nematodes/plant, but not at 300 nematodes/plant, and that *C. echinospermum* was more sensitive to the initial nematode density, because it responded more steeply to the 150-225 change ($P = 0.087$). Thus, *C. echinospermum* had lower final nematode counts than the other two species at 150 nematodes/plant, while at 225 and 300 nematodes there is no significant differences observed. Therefore, the data indicate that 225 nematodes is the optimal level. Development population density of *P. thornei* was different in the inoculation density of 150 nematodes in all species. It means the population density of nematodes changed in all species when initial inoculum density was 150 nematodes/plant. In order, the highest population density of *P. thornei* in the inoculation density of 150 nematodes was observed in *C. arietinum* (Gökçe MR = 3, Çagatay MR = 2.9 and Menemen MR = 2.8) and the lowest population density observed in *C. echinospermum* (Karabahçe MR = 2.5, Destek and Ortance MR = 2.6) (Figure 2). Also, there was no significant difference between *Cicer* species and species with an initial inoculation density of 225 and 300 nematodes except initial inoculum density of 150 nematodes. Likewise, there was no difference observed in the population density of *P. thornei* in the inoculation density of 225 and 300. Also, the final nematode counts rose to a peak at 225 nematodes/plant in all species, with no change as the population rises to 300 nematodes/plant (Figure 2).

Table 1. Analysis of variance of nematode multiplication rates of accessions and their interactions

Period. Density. Rep stratum	Source of variation*				
	d.f.	s.s	m.s	v.r	F pr
Period	1	0.7241	0.7241	0.57	0.458
Density	2	14.1543	7.0771	5.62	0.013
Lin	1	11.2589	11.2589	8.94	0.008
Quad	1	2.8954	2.8954	2.30	0.147
Period.Density	2	0.4220	0.2110	0.17	0.847
Period.Lin	1	0.3451	0.3451	0.27	0.607
Period.Quad	1	0.0769	0.0769	0.06	0.808
Residual	18	22.6785	1.2599	8.86	
Period.Density.Rep.*Units* stratum					
Species	2	0.3471	0.1735	1.22	0.298
Period.Species	2	0.6081	0.3041	2.14	0.122
Density.Species	4	0.8025	0.2006	1.41	0.234
Lin.Species	2	0.7065	0.3533	2.48	0.087
Quad.Species	2	0.0960	0.0480	0.34	0.714
Species.Coll_site	4	0.2728	0.0682	0.48	0.751
Period.Density.Species	4	0.4039	0.1010	0.71	0.587
Period.Lin.Species	2	0.0734	0.0367	0.26	0.773
Period.Quad.Species	2	0.3305	0.1653	1.16	0.316
Period.Species.Coll_site	4	0.0684	0.0171	0.12	0.975
Density.Species.Coll_site	8	0.2868	0.0358	0.25	0.980
Lin.Species.Coll_site	4	0.0992	0.0248	0.17	0.951
Quad.Species.Coll_site	4	0.1876	0.0469	0.33	0.858
Species.Coll_site.Var	2	0.4048	0.2024	1.42	0.244
Period.Density.Species.Coll_site	8	0.7664	0.0958	0.67	0.714
Period.Lin.Species.Coll_site	4	0.3056	0.0764	0.54	0.709
Period.Quad.Species.Coll_site	4	0.4608	0.1152	0.81	0.521
Period.Species.Coll_site.Var	2	0.4120	0.2060	1.45	0.238
Density.Species.Coll_site.Var	4	1.0689	0.2672	1.88	0.117
Lin.Species.Coll_site.Var	2	0.4619	0.2310	1.62	0.201
Quad.Species.Coll_site.Var	2	0.6070	0.3035	2.13	0.122
Period.Density.Species.Coll_site.Var	4	0.6446	0.1612	1.13	0.344
Period.Lin.Species.Coll_site.Var	2	0.1766	0.0883	0.62	0.539
Period.Quad.Species.Coll_site.Var	2	0.4681	0.2340	1.64	0.197
Residual	144	20.4873	0.1423		
Total	215	64.5524			

* df: contains degree of freedom which are measure of how much information is contained in each variance;
s.s: Means squares, which are calculated by multiplying the mean square and degree of freedom in the same row;
ms (Means squares): The variance between treatment;
v.r: The ratio of the between treatment variance to the within treatment variance;
F pr or P value: Significance value $P < 0.001$.

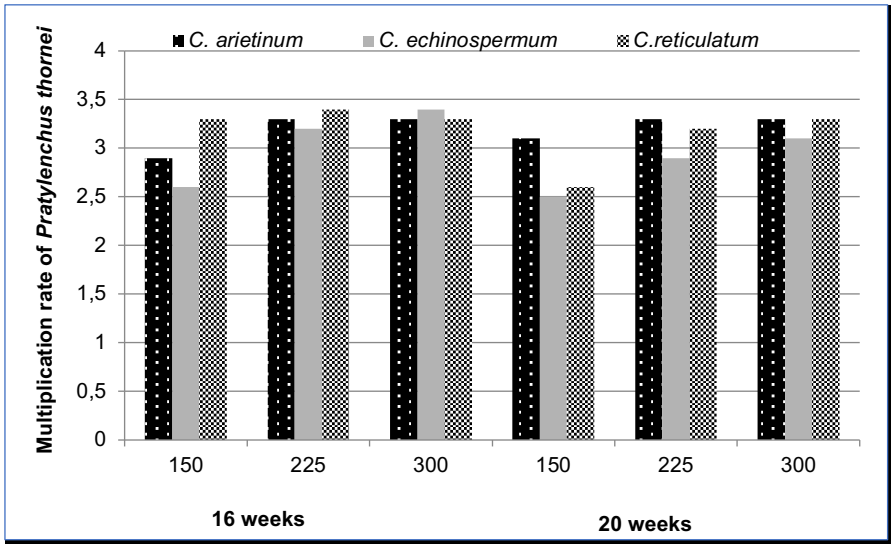

Figure 1. Multiplication rate of *Pratylenchus thornei* on *Cicer* species at two harvest time.

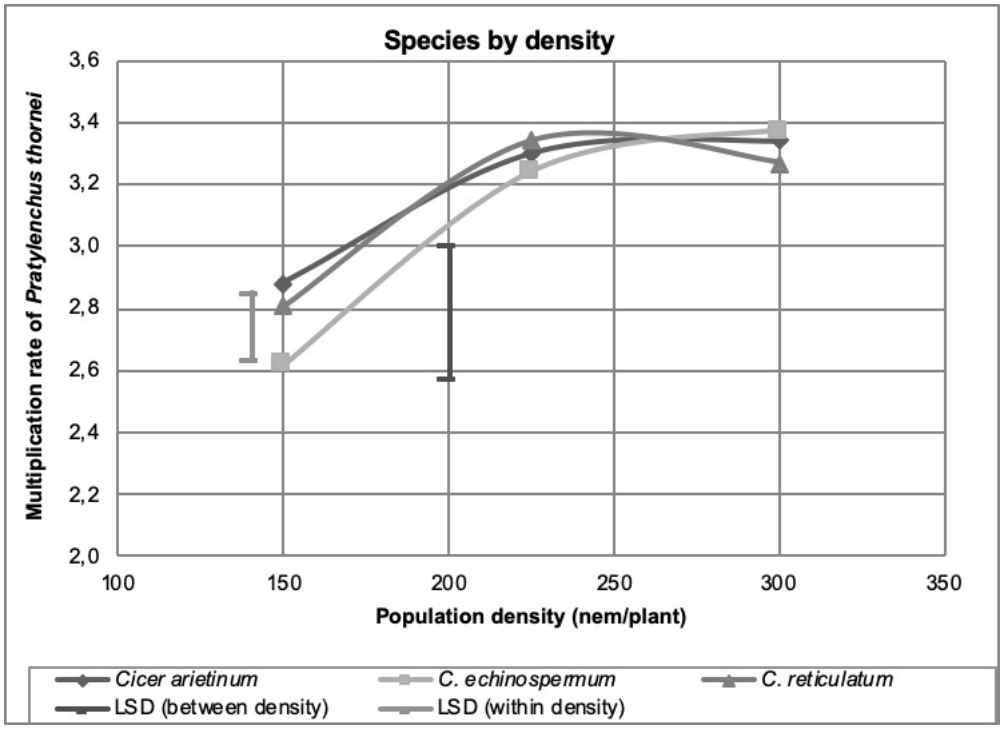

Figure 2. Multiplication rate of *Pratylenchus thornei* on chickpea species at different initial nematode densities.

The reproduction factor of *P. thornei* among chickpea genotypes is shown in Figure 3. The initial density of *P. thornei* clearly had an effect on nematode multiplication and that there is some indication of an interaction between species and the linear effect of nematode density. Statistically significant differences among cultivars with 150 nematodes/tube indicated that *C. echinospermum* has lower nematode multiplication than the other *Cicer* species, whereas at 225 and 300 nematodes/tube there is no changing observed among chickpea genotypes. Also, the population densities of *P. thornei* in plots of *C. arietinum* and *C. reticulatum* were more than *C. echinospermum* at 225 and 300 nematodes/tube. In order, among the *C. arietinum* and *C. reticulatum* genotypes, population development of *P. thornei* at Menemen and

Kalkan plots was less than Şırnak and Gökçe plots (Figure 3). Both were similarly responsive to *P. thornei* and, development of populations was more than any *C. echinospermum* genotype. Also, the relationships between wild and domesticated *C. arietinum* indicated that multiplication rate of *P. thornei* at wild *Cicer* spp. plots (*C. reticulatum* and *C. echinospermum*) were statistically significantly less than domesticated cultivars (*C. arietinum*).

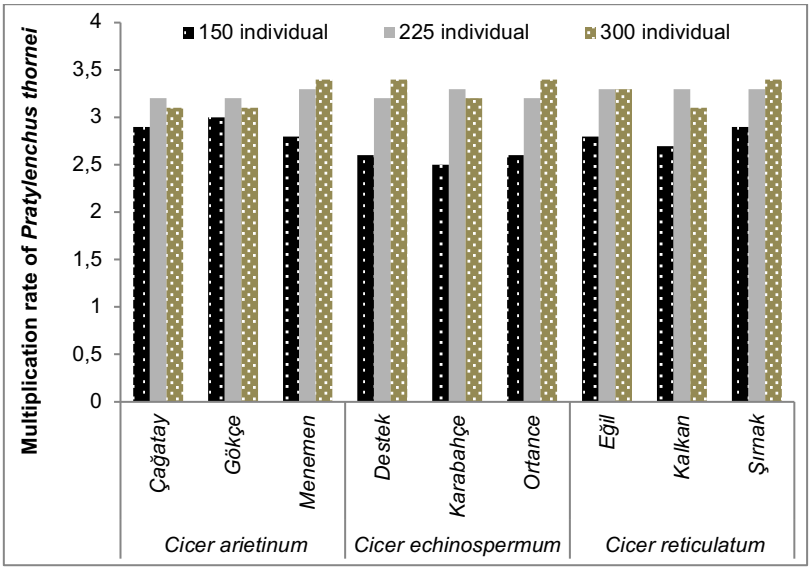

Figure 3. Nematode multiplication ratio of the chickpea cultivars in different initial inoculum densities of *Pratylenchus thornei*.

Discussion

Factors such as soil texture, soil temperature application technique, growing time and relationship between initial and final population density of plant can affect the population density of nematodes (Seinhorst, 1965; Oostenbrink, 1966; Toktay et al., 2012). These factors need to be carefully optimized. Other factors can also have an effect on the presence or absence of nematodes in the soil at given times during the growing time of a crop. Also, determination of the relationship between the initial and final population density of nematodes to keep the nematode population below the damage threshold level is important in chickpea breeding programs. Schomaker and Been (2006) suggested that the information on initial population density and determination of damage level under specific conditions for specific crops are essential for nematode pest management programs.

According to the results of this screening study with limited accessions of *Cicer* species, *C. echinospermum* was more resistant to *P. thornei* than *C. arietinum* and *C. reticulatum*. In other words, *C. arietinum* and *C. reticulatum* genotypes were the most susceptible to *P. thornei*. Both *C. arietinum* and *C. reticulatum* genotypes responded similarly to *P. thornei*, and were more susceptible than any *C. echinospermum* genotype. A similar study by Thompson et al. (2011) indicated that there were species differences in nematode sensitivity and multiplication of root lesion nematodes has been found to be different for each *Cicer* species.

The statistically significant difference of nematode multiplication between wild and domesticated genotypes indicated that wild species (*C. reticulatum* and *C. echinospermum*) were more resistant to *P. thornei* than domesticated *C. arietinum*. Singh and Ocampo (1997) reported that the use of *C. echinospermum* and *C. reticulatum* from the wild gene pool for chickpea is a practical option.

In this study, an efficient optimized method was developed for screening chickpea cultivars to *P. thornei*. In conclusion, this study indicated that inoculation each plant with an initial density of 225 nematodes/tube was the best selection to test chickpea cultivars for resistance to *P. thornei*. Similarly, Toktay et al. (2012) compared nematode inoculum density in wheat and reported that the best-inoculating density was 400 because their study did not find a significant difference between 400 and 600 nematodes. For chickpea, an optimized screening method has been successfully developed. It allows the identification of resistance against the root lesion nematode, *P. thornei* at harvesting time and can be useful to the study of resistance mechanisms. Also, the results indicated that there was no change in the population density of *P. thornei* during the growing time (16 and 20 weeks) in *Cicer* species. Singh and Ocampo (1997) noted that the chickpea is a cool-season crop and required the growing time of 100 d to reach maturity. Reen & Thompson (2009) reported that a longer growth period was required to maximize differences in *P. thornei* densities between cultivars of chickpea (18-20 weeks) than in wheat (16-18 weeks) under laboratory conditions. Taylor et al. (2000) showed that the mean final population density of *P. neglectus* of twenty wheat genotypes was lower than that of six chickpea genotypes 21 or 26 weeks after sowing.

The optimized screening technique will be useful to test chickpea genotypes for resistance to *P. thornei* under laboratory conditions. This study is the first to assess chickpea genotypes collected from Turkey for resistance to *P. thornei*, some of which offer new sources of *P. thornei* resistance and genetic diversity useful for international chickpea breeding programs.

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