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The Effects of Root Lesion Nematodes (*Pratylenchus thornei*) on Rhizobium Bacteria of Chickpea Plant

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ABSTRACT

Chickpea (*Cicer arietinum*) is one of the most significant legume crops and supply high-quality protein for human nutrition.Legume crops such as chickpea are important agriculturally because of their symbiotic ability to nitrogen fixation with specific soil bacteria. Legumes like chickpea depend on nitrogen provided by the activity to grow, but these rhizobium bacteria are affected by plant-parasitic nematodes that cause less activity and decrease the number of nodules in chickpea plant. The root lesion nematodes (*Pratylenchus thornei*) are common and economically important pests described as one of the limiting factors in agriculture and the growing chickpea field in the world. In this study, the effects of this nematode on the number of rhizobia (nodules) and rhizobium bacteria activity were assessed in both wild and domesticated accession of *Cicer* species under laboratory conditions. We inoculated all *Cicer* accession with the *Mesorhizobium* bacteria and with one species of the genus *Pratylenchus* (*P. thornei*). The result showed that *P. thornei* has a negative impact on the number of nodules and the activity of rhizobium bacteria. Nematode infection on chickpea caused decreased nodulation. Overall, nematode infected plant formed 4-8 nodules/root and less nodule number than an uninfected plant.

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ÖZET

Nohut (*Cicer arietinum*) en önemli baklagil bitkilerinden biridir ve insan beslenmesi için yüksek kaliteli protein sağlar. nohut gibi baklagil bitkileri, spesifik toprak bakterileriyle azot fiksasyonuna yönelik simbiyotik yetenekleri nedeniyle tarımsal açıdan önemlidir. Nohut gibi baklagiller büyüme aktivitesi sağladığı nitrojene bağlıdır, ancak bu rizobium bakterileri nodül bitkilerinde daha az aktiviteye neden olan ve nodül sayısını azaltan bitki paraziti nematodlardan etkilenir. Kök lezyon nematodları (*Pratylenchus thornei*), dünya tarımında büyüyen nohut tarlalarında en önemli sınırlayıcı faktörlerden biri olarak tanımlanan yaygın ve ekonomik açıdan önemli zararlılardır. Bu çalışmada, bu nematodun rizobium (nodül) sayısı ve rhizobium bakteri aktivitesi üzerindeki etkileri, laboratuvar koşullarında *Cicer* türlerinin hem yabani hem de yerli genotiplerinde değerlendirilmiştir. Tüm nohut genotipleri Mesorhizobium bakterileri ve *Pratylenchus* cinsinin bir türü (*Pratylenchus thornei*) ile bulaştırılmıştır. Sonuç olarak *P. thornei*'nin nodül sayısı ve rhizobium bakterilerinin aktivitesi üzerinde olumsuz bir etkisi olduğunu göstermiştir. Nematod enfeksiyonu, nohutta nodülasyon sayısının azalmasına neden olmuştur. Nerdeyse, nematod ile enfekte olmuş bitki, enfekte olmayan bitkiye göre 4-8 arası az nodül sayısı oluşturmuştur.

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INTRODUCTION

Chickpea (*Cicer arietinum* L.) is one of the most important plants between legumes crops in the world. The most important chickpea production areas in the world are India, Australia, Myanmar, Ethiopia, Turkey, Pakistan, Russia, Iran, Mexico, USA, and Canada (FAO, 2017).

Almost all species want a symbiotic relationship with other species to assess easily carry out the crucial biological activity (Shapira, 2016). Legumes crop can make a symbiotic relationship with nitrogen-fixing soil bacteria that is called rhizobia bacteria. This symbiosis relationship causes to form nodules on the plant root by this bacteria that convert atmospheric nitrogen into ammonia that can be used by the plant. Also, the advantage result of symbiotic nitrogen fixation by chickpea nodules and rhizobium bacteria can make the facility of growing the crop in many nitrogen-poor soils (Carter et al., 1994). Most of the plants depend on symbiotic bacteria or fungi to grow, and on their activity for reproduction (Friesen, 2013; Busby et al., 2017). The fixation of nitrogen by legumes plays an important role in agricultural production. Legumes crops such as chickpea are partners with rhizobium bacteria for the fixation of nitrogen.

The average chickpea yield in the world is changing because of many biotic plant-parasitic nematodes and abiotic soil factors that can cause an important reduction in grain quantity and quality of chickpea (Singh et al., 1994; Sudupak et al., 2002). Castillo and Vovlas (2007) indicated that the plant-parasitic nematodes are one of the most economically and important pests affecting chickpea as the biotic factors. Parasites can cause important changes in the symbiotic relationship, usually in two ways (Strauss and Irwin, 2004). Sasser and Freckman (1987) indicated that annual yield losses of the total global production of chickpea was 14% by the effect of plant-parasitic nematodes. Different species of nematode cause important economic damage in chickpea. Among these nematodes, root-lesion nematodes (*P. thornei*) is one of the most important species. Ballhorn et al. (2014) showed that the activity of symbiotic partnerships and formed rhizobium associations was decreased because of this parasite in the infected host plants. The symbiotic relationship between rhizobia and plant genotype together can affect the yield potential of chickpea and the symbiosis response to biotic and abiotic stresses (Correa and Barneix, 1997). Hussey et al. (1976) observed that plant-parasitic nematodes can affect symbiotic nitrogen fixation on legumes roots by Rhizobium bacteria. Also, the study by Vovlas et al. (1998) showed that plant-parasitic nematodes (*Meloidogyne incognita*) affected more than 25% of *Mesorhizobium* bacteria activity in the chickpea nodule.

The aim of study was to know about the interaction activity between legumes and nitrogen-fixing bacteria (rhizobia), and how do parasitic nematodes can affect the rhizobium bacteria activity. For this purpose, we inoculated all *Cicer* genotypes with one of the root-lesion nematodes (*Pratylenchus thornei*). Using the number of nematodes was to estimate the damage caused by *P. thornei* in chickpea, we observed that chickpea genotypes differed in susceptibility to nematode infection, and between the number of rhizobia (nodules) and nematode number differed in infectivity.

The number of nodules plays an important in the fixation of nitrogen and agricultural production by legumes. Many legume crops are infected by plant-parasitic nematodes causing photosynthates damage (Dhandaydham et al., 2008; Goverse and Smant, 2014).

This research provides scientific information to understand how root-lesion nematodes (*P. thornei*) can impact the number of nodules and activity of the rhizobium bacteria in the infected chickpea plant. Because nodule number is correlated with rhizobium fitness in legumes and also it can provide the benefit as a proxy for plant partner quality in the rhizobium symbiotic (Heath and Tiffin, 2009).

MATERIALS and METHODS

In this study, 9 accessions of Turkish domesticated and wild *Cicer* species 3 *Cicer arietinum* (Azkan, Çagatay, and Gökçe), 3 *Cicer echinospermum* (Karabahçe, Ortace, and Destek), and *3 Cicer reticulatum* (Şırnak, Kallen and Eğil) were used to test how root-lesion nematodes (*P. thornei*) impact the number of nodules (rhizobium) under laboratory conditions. For this purpose, we compared the nodule number and total nodules between nematode-infected and uninfected plants.

Cicer genotypes using in this study were collected from 3 collection sites of Şanliurfa, Şirnak, and Diyarbakir province of Turkey. The collection seeds were sacrificed by making a small cut in the seed coat before germinating to improve water absorption and germination in the wild *Cicer* spp. The individual chickpea seeds were disinfected with hypochlorite (4%) and alcohol (30%). Moreover, to enhance seed germination, about 30 seeds of each accession were placed on the surface of wet filter paper at 4°C for 3 days in sterile Petri dishes (Garcia et al. 2006). We incubated seeds at room temperature for 16 hours before planting.

Germinated seeds were then planted in the open-ended standard small tube (16 cm in high 2.5 cm in diameter) that contained 60 gr field soil, (73% clay, 16.5% silt, and 10% river sand) and supported by a box frame. The soil inside the tube was sterilized by an autoclave machine for 5 minutes at 121°C before sowing the seeds and keep in the laboratory at the University of Cukurova at 25°C, on a 16:8 light: dark cycle.

Isolation and culture of nematode

Soil and root samples were collected from chickpea fields between June and July of 2019. Nematodes were extracted by using the Baermann funnel technique in the laboratory (Hooper, 1986). Each root and soil sample consisted of 5- 10 samples (taken at depth of 5-10 cm), with the final weight of 1-2 kg soil per sample. Root-lesion nematodes (*Pratylenchus thornei*) used in this study were cultured on carrot cultures by using the method described by Nicol and Vanstone (1993). This nematode was collected originally from growing chickpea regions in Şanliurfa province (Harran district) located in the Southeastern Anatolia Region of Turkey and cultured in the nematology laboratory at Çukurova University.

Isolation and culture of bacteria

Rhizobia were isolated from nodules of chickpea from field-grown chickpea from regions in Şanliurfa province (Harran district) located in the Southeastern Anatolia Region of Turkey. For rhizobia isolation (*Mesorhizobium*), 2–11 nodules from chickpea and a maximum of five nodules/plant from field-grown chickpea were selected. The rhizobia from the selected nodules were isolated and cultured following standard protocols using CRYEMA (yeast extract mannitol agar medium with congo red) described in Somasegaran and Hoben (1994).

Two weeks after germination, each chickpea plant was inoculated with 1 ml of Mesorhysobium culture and 1 ml of nematode consisting of 225 *P. thornei* (Behmand et al., 2019). Nematodes and rhizobia were inoculated onto the plant at the same time to prevent effects associated with different arrival times. The plants were harvested 16 weeks after planting and the number of nodules and on each root system were counted under a dissecting microscope.

Statistical analyses

The data were analyzed using a randomized block design (One-way ANOVA) in Genstat (V13). These analyses included treatment (nematode presence or absence) and random effects of genotype, block, treatment × genotype, and treatment × block. Significant differences between treatment and replication of data were calculated at P< 0 .001. Outliers and variance distribution was assessed using residual plots. Data were transformed as necessary.

RESULTS AND DISCUSSION

The result showed that *P. thornei* has a negative impact on the number of nodules and the activity of rhizobium bacteria. Seed inoculation with *Mesorhyzobium* bacteria and *P. thornei* had a significant effect on the number of formed nodules (rhizobium). The average nodule number in the inoculation seed with *Mesorhyzobium* bacteria was more than inoculation seed with *Mesorhyzobium* bacteria and *P. thornei* (Figures 1 and 2).

There was a significant difference observed between root-lesion nematodes (*P. thornei*) and the number of nodules produced in the absence of nematodes P<.001 (Table 1). Nematode-infected plants produced fewer nodules than uninfected plants (Figures 1 and 2). Where, nematode infected plant formed 4-8 nodules/root and less nodule number than the uninfected plant. The frequency distribution of nodules on the infected and uninfected plant with nematode is shown in figure 1.

Also, the results of the study indicated that the number of nodules (rhizobium) not only was different between legume species even cultivars within a species were different. There was a significant effect of *Cicer* cultivars for total nodule and the ability of them to attract rhizobia was changed between *Cicer* species P<0.001 (Table 1). The number of nodules among the cultivars studied, 'Çagatay', 'Gökçe', and Menemen was higher than other cultivars. These effects showed that plant genotypes differed in how nodule traits were impacted by nematode infection. A similar study by Bhuiyan et al. (2008) and Sattar et al. (1998) showed that genotypic diversity affected the number of nodules in both wild and domesticated chickpea and other legumes crops.

In addition, results demonstrated that the maximum of nodule numbers among the cultivars of *C. arietinum* was higher than other *Cicer* species and there was a significant difference observed between *Cicer* species for the nodulation potential P<.001 (Table 1 and Figure 3), but the negative effect of nematode on rhizobium (nodules) in the wild *Cicer* species (*C. echinospermum* and *C. reticulatum*) was less than *C. arietinum* (Figures 2 and 3).

Both wild *Cicer* sp. have a similarly responsive to *P. thornei* and the performance of them for the nodulation was better in nematode infected plant than any *Cicer arietinum* cultivars (Figure 2). However, among the *C. echinospermum* and *C. reticulatum* cultivars, Karabahce, Ortace, and Destek have given a good performance to *P. thornei* than any *C. reticulatum* cultivars (Figures 2 and 3). Both bacterial and plant genes can affect the development of root nodules (Buttery et al., 1997 and Danso et al., 1987). Therefore, any changes in these organisms cause to decrease the development of the nodules and therefore affect nitrogen fixation (Rupela and Saxena, 1987).

Table 1. Analysis of variance
Çizelge 1. Varyans analizi

Source of variation	Degree of Freedom (df)	Sum Square (SS)	Mean square (MS)	F Ratio	P Value
treatment	1	387.347	387.347	47.51	<.001
species	2	895.194	447.597	54.90	<.001
Treatment xspecies	2	48.694	24.347	2.99	0.057
Residual	66	538.083	8.153		
Total	71	1869.319			

* 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.

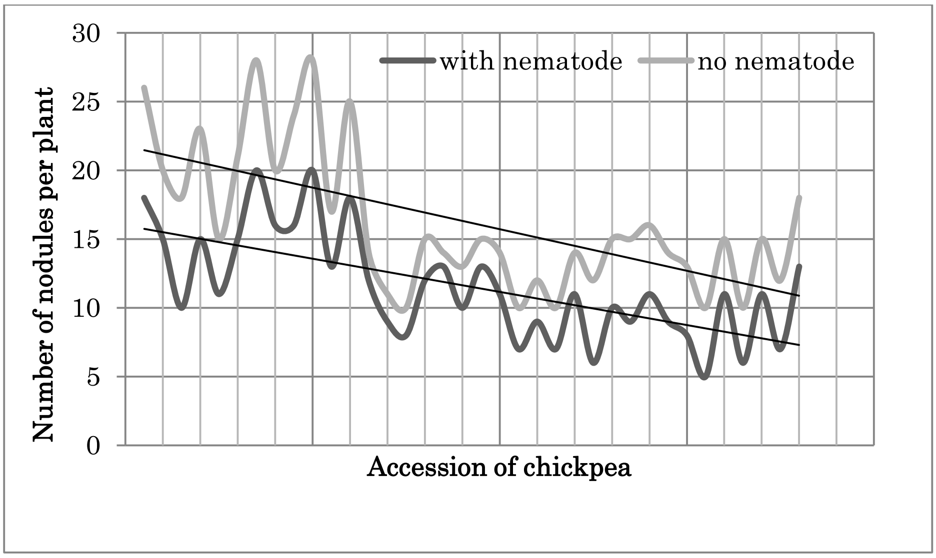

Figure 1. Frequency distribution of nodules on the infected and uninfected plant with nematode
Şekil 1. Nematod ile enfekte ve enfekte olmayan bitki üzerindeki nodüllerin frekans dağılımı

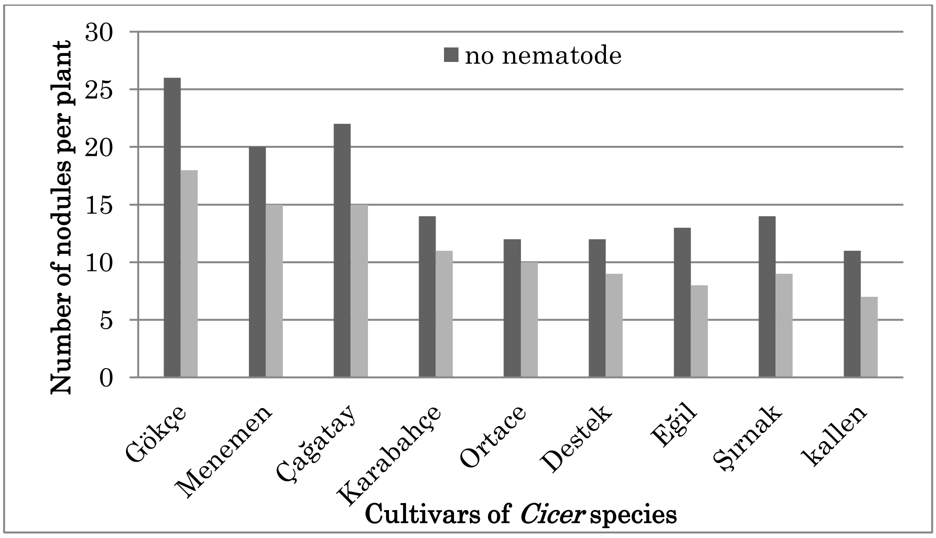

Figure 2. The effects *of Pratylenchus thornei* on the number of nodules between *Cicer* cultivars.
Şekil 2. *Pratylenchus thornei*'nin nohut çeşitleri arasındaki nodül sayısı üzerine etkileri.

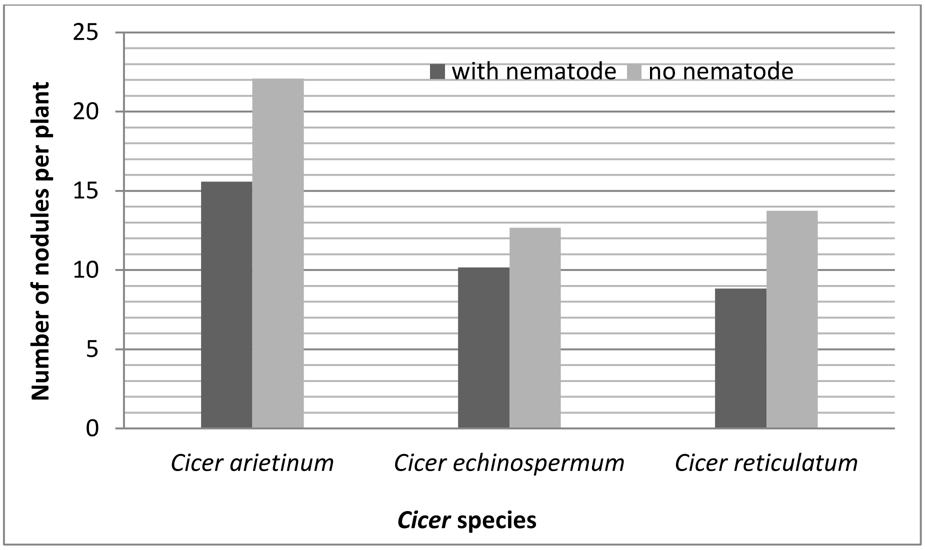

Fig 3. The effects of *Pratylenchus thornei* on the number of nodules between *Cicer* species.
Şekil 3. *Pratylenchus thornei*'nin nohut türleri arasındaki nodül sayısı üzerine etkileri.

According to the study showed that biotic factors such as plant-parasitic nematodes disrupt the symbiotic relationship between leguminous plants and nitrogen-fixing rhizobia bacteria. In this study chickpea that were infected by parasitic nematodes (*P. thornei*) formed fewer total nodules than uninfected plants. The number of nodules in the accession of domesticated *Cicer* with rhizobium bacteria had a more nodules number than wild genotype species. However, plants that formed more nodules with rhizobia were more attack and heavily infected by nematodes. *Mesorhyzobium ciceri* that infects the chickpea is specific and rarely present in the soil in which the chickpea is not recently grown (Elhadi and Elsheikh, 1999). Also, Abdalla et al. (2011) indicated that the number nodulation of the chickpea and the success of bacterial inoculation increased in the presence of the native bacterial population.

The study showed that the symbiotic relationship between rhizobium and plants is negatively affected by parasite infection. Also, in the presence of nematodes, plants formed fewer or prevent associations with symbiotic rhizobia. We found that nematode-infected plants formed 29% fewer total nodules on root than uninfected plants. A similar study by Wood et al. (2018) showed that *Meloidogyne hapla* nematode has a negative impact on the number of nodules and formed 23% fewer nodules and 19% less total nodule biomass per gram in Medicago plant roots.

The effect of nematode on nodule number is different between *Cicer* species. Moreover, while some *Cicer* genotypes formed fewer nodules when infected by nematodes (*C. arietinum*), others including *C. echinospermum* and *C. reticulatum* genotypes were not more affected. In this study, the relationships between wild and domesticated *Cicer* sp. indicated that negatively affected of *P. thornei* on rhizobium in both wild *Cicer* sp. (*C. reticulatum* and *C. echinospermum*) were less than domesticated varieties (*C. arietinum*).

The useful effect of rhizobia symbiosis on legume crops nutrition and growth and the association of rhizobia with plant-parasitic nematodes in the rhizosphere cause investigations into the potential role of nematode parasitism on nodulation, and accordingly on symbiotic nitrogen fixation. Future studies in the effect of root-lesion nematodes on rhizobium should explore the genetic capacity is the influences the degree of these relationships or not. Wood et al. (2018) demonstrated that the effect of nematode on crop plants is different because of genotype variation in the plant. Burghardt et al. (2017) also found significant genotype variation between plant genotypes in the definition of defense genes in nodules. The information provided by these results will be useful for the clarification of nematodes, root-nodule bacteria, and host plant relations, but more studies are needed to test the effect of root-lesion nematodes on rhizobium for developing chickpea breeding programs to keep the economic damage below the threshold level.

CONCLUSION

It is concluded that root-lesion nematodes (*P. thornei*) infection can impact the number of nodules in the chickpea plant. Disease management by using resistance cultivars is suitable to be used for control of root lesion nematodes and lessen the negative impact on the number of nodules and the activity of rhizobium bacteria.

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Statement of Conflict of Interest

Authors have declared no conflict of interest.

Author's Contributions

TB designed the study, conducted the experiments, and prepared the manuscript. IHE provided technical guidance, and critically revised the manuscript for intellectual content. Two authors read and approved the final manuscript.

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