

**ÇUKUROVA UNIVERSITY
INSTITUTE OF NATURAL AND APPLIED SCIENCES**

PhD THESIS

Tohid BEHMAND

**SCREENING OF CHICKPEA GENOTYPES COLLECTED
FROM TURKEY AGAINST TO THE ROOT LESION
NEMATODES, *Pratylenchus neglectus*, *Pratylenchus thornei* AND
Ditylenchus dipsaci FOR RESISTANCE**

PLANT PROTECTION DEPARTMENT

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PLANT PROTECTION DEPARTMENT**

This thesis was accepted by the following jury members' unanimity / majority vote on 01.07.2018.

.....
Prof. Dr. Ibrahim Halil ELEKCİOĞLU
Supervisor

.....
Prof. Dr. Ekrem ATAKAN
Jury

.....
Prof. Dr. Celaleddin BARUTÇULAR
Jury

.....
Prof. Dr. Ramazan CANHİLAL
Jury

.....
Prof. Dr. Ramazan ÇETİNTAŞ
Jury

**This thesis was applied from the plant protection department.
Code:**

**Prof. Dr. Mustafa GÖK
Director of the Institute**

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ABSTRACT

PhD THESIS

SCREENING OF CHICKPEA GENOTYPES COLLECTED FROM TURKEY AGAINST TO THE ROOT LESION NEMATODES, *Pratylenchus* *neglectus*, *Pratylenchus thornei* AND *Ditylenchus dipsaci* FOR RESISTANCE

Tohid BEHMAND

CUKUROVA UNIVERSITY INSTITUTE OF NATURAL AND APPLIED SCIENCE DEPARTMENT OF PLANT PROTECTION

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: Prof. Dr. Celaleddin BARUTÇULAR
: Prof. Dr. Ramazan CANHİLAL
: Prof. Dr. Ramazan ÇETİNTAŞ

The root lesion nematodes (*P. thornei*, *P. neglectus*) and *D. dipsaci* are economically impacting pests described as one of the major limiting factors in chickpea production. These nematodes have shown to cause yield losses and reduce the number of nodules in the root system of chickpeas. One of the major strategies is to develop resistance to root lesion nematodes and to *D. dipsaci* by accessing and exploiting their natural variation and resistant cultivars. In this study, more than 400 accessions of wild *Cicer* sp (*Cicer reticulatum* and *Cicer echinospermum*) were tested against the root lesion nematodes (*P. thornei*, *P. neglectus*) and *D. dipsaci* for resistance in a laboratory and field experiment. The comparing to the field study, laboratory study was very insightful in verifying the field results. A very small subset of the collection was evaluated in the field experiment, where nematode populations were smaller than those recorded in the laboratory experiment and indicated that there was a correlation between susceptible and resistant lines identified in field and laboratory experiments. The main aim of this study was to find the most appropriate initial nematode density, to determine the most suitable harvest time during an optimization study while identifying the geographical distribution of the most important plant-parasitic species, and evaluating the population dynamics of RLN to determine chickpea varieties with resistance to nematodes. The result of this plant-parasitic nematodes associated with the chickpea study indicated that several nematodes were commonly associated with chickpea in Turkey and root lesion nematodes were present in all sampled fields. Moreover, the result of the laboratory and field study indicated that the wilds *Cicer* spp (*C. reticulatum* and *C. echinospermum*) were more resistant to nematodes than domestic (*C. arietinum*) and indicating there was a very strong interaction between nematode species within *Cicer* species ($P<0.001$).

ÖZET

DOKTORA TEZİ

Bazı Nohut Çeşitlerinin Kök Yara Nematodları (*Pratylenchus thornei*, *Pratylenchus neglectus*) ve Soğan Sak Nematodu (*Ditylenchus dipsaci*)'na Karşı Dayanıklılıklarının Belirlenmesi

Tohid BEHMAND

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: Prof. Dr. Celaleddin BARUTÇULAR
: Prof. Dr. Ramazan CANHİLAL
: Prof. Dr. Ramazan ÇETİNTAŞ

Kök lezyon nematodları (*P. thornei*, *P. neglectus*) ve soğan sak nematodu (*D. dipsaci*), nohut üretiminde başlıca sınırlayıcı faktörlerden sayılan ekonomik oneme sahip zararlıların başında gelmektedir. Bu nematodların verim kaybına neden olduğu ve nohuttaki nodül kök sisteminin sayısında azalmaya neden olduğu gösterilmiştir. Bu nematodlara karşı direnç geliştirmeye yönelik başlıca stratejilerden biri, doğal varyasyonlarına ve dayanıklı çeşitliliğe ulaşmak ve bunları kullanmaktır. Bu çalışmada, 400'den fazla yabancı *Cicer* sp. (*C. reticulatum* ve *C. echinospermum*) çeşidi laboratuvar ve arazi çalışmasında kök lezyon nematodlarına (*P. thornei*, *P. neglectus*) ve *D. dipsaci*'ye karşı dayanıklılıkları araştırılmıştır. Arazi çalışmalarının laboratuvar çalışmasından daha etkili olduğu görülmüştür. Nematod popülasyonlarının laboratuvar çalışmasında kaydedilenlerden daha küçük olduğu tarla çalışmasında koleksiyonun küçük bir alt kümesi değerlendirilmiş ve tarla ve laboratuvar çalışmasında tanımlanan hassas ve dirençli hatlar arasında bir korelasyon olduğunu görülmüştür. Bu çalışmanın temel amacı, en iyi başlangıç nematod yoğunluğunu, en iyi hasat zamanını, en önemli bitki-parazitik türlerin coğrafi dağılımını belirlemek, nohutta nematodlara dirençli bitkileri ve çeşitleri belirlemek için RLN popülasyon dinamiklerini değerlendirmek olmuştur. Nohut çalışmasıyla ilgili bitki parazit nematodlarının Türkiye'de nohutta olduğu ve bunların arasında tüm örneklenmiş alanda kök lezyon nematodun yaygın bulunduğu ve nohutta düşük verime neden olduğun görülmüştür. Ayrıca, laboratuvar ve arazi çalışmasının sonuçları, *Cicer* sp (*C. reticulatum* ve *C. echinospermum*) 'un, nematodlara (*C. arietinum*) göre daha dirençli olduğunu ve nohut türleri içinde nematod türleri arasında çok güçlü bir etkileşim olduğu gözlemlenmiştir $P < 0.001$.

Anahtar kelimeler: Direnç, Nohut, *Pratylenchus thornei*, *Pratylenchus neglectus* ve *Ditylenchus dipsaci*.

SUMMARY

Chickpea plants are constrained by limited genetic and adaptive diversity. Widening this diversity is the focus of an international collaboration that collected, phenotyped and introgressed wild cicer into chickpea including institutes of Turkey. Before starting this study, a yield loss caused by root lesion nematodes (*P. thornei*, *P. neglectus*) and *D. dipsaci* were observed. The main objectives of this study were to assess the sampling procedure, to evaluate the extent and importance of yield losses caused by lesion nematodes (*P. thornei*, *P. neglectus*) and *D. dipsaci*, to estimate the population dynamic of RLN and *D. dipsaci* in legumes (chickpea), to detect whether differentials occur in field crops, to identify whether resistance occurs between geographically isolated of population density of *P. thornei* and to optimize the screening study of chickpea varieties inoculated with *P. neglectus*, *P. thornei* and *D. dipsaci* to find the best initial nematode density and harvest time.

The optimization study also was conducted on the closely related species, *P. neglectus* including, the differential between of population density and effect of this nematode species on chickpea yield. The other main aim of this study was to widen the genetic and adaptive diversity of chickpea by collection, characterization, evaluation, and exploitation of wider cicer genetic resources. Also, to find the best sampling procedures for RLN and *D. dipsaci*, the extraction method from plant and soil was assessed.

To get an understanding of the population density of nematodes, experiments were conducted in growth room and by growing chickpeas in fields large pots. For the laboratory study, each accession of the wild *Cicer* spp. (*C. arietinum*, *C. reticulatum*, and *C. echinospermum*) were sown into an infected tube (produced by the addition of infected carrot callus culture and infected chickpea roots and soil exam). Also, in contrast, the initial densities of both of *P. thornei*, *P. neglectus* and *D. dipsaci* were conducted in two chickpea fields. At both

experiment sites (Ankara and Sanliurfa), a close relationship was indicated between an initial density of nematodes and multiplication rate (P_f/P_i) and between initial density and yield for an intolerant chickpea grown across these plots.

Yield losses of field caused by nematodes were also indicated that there was a correlation of population density of lesion nematodes and *D. dipsaci* with the crop yield. Over 400 accessions of wild both *Cicer* species (*C. reticulatum* and *C. echinospermum*) were assessed for resistance to *P. thornei*, *P. neglectus* and *D. dipsaci* under laboratory conditions. Also, more than 400 accessions of wild *Cicer* spp. were evaluated for resistance to nematodes in the field.

As resistance is explained by the ability to reduce multiplication of nematodes, the assessment of nematodes from soil after harvest was assumed to be closely related to resistance. The changes observed in multiplication rates and final population densities were dependant on seasonal conditions at both sites and also possibly variations between population density of *P. thornei*. The genetic differences between the changes in nematode population density and phenotypic differences evaluated by the reproduction rate of nematodes in legumes in grow room laboratory tests supported this.

The results of this study reports that *P. thornei*, *P. neglectus*, and *D. dipsaci* were significant pathogen in agricultural regions, especially in the chickpea growing fields of Turkey. The broad range of these nematodes within field crops were confirmed, but the tolerance or resistance to accessions and varieties was determined and can be used within cereal cropping rotation to reduce the economic effect of this species.

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LIST OF ABBREVIATIONS

Pi	: initial population
Pf	: final population
Pf/Pi	: multiplication rate of nematode
ml	: milliliter
ha	: hectare
kg	: kilogram
g	: gram
t	: tonne
m.s	: mean squared
s.e.d	: standard error of means
s.e	: standard error
ns	: not significant
Rep.	: replicate/replication
n	: number
cm	: centimetre
m	: metre
Sp.	: species
Spp.	: species (plural)
L.S.D	: least significant difference
°C	: degree celsius
h	: hour
RLN	: root lesion nematodes



1. GENERAL INTRODUCTION

Different types of pests and diseases can attack agriculture crops, cause significant problems and decrease crop yields. Argios (2005) reported that plant parasitic nematodes, among other main plant pathogenic microorganisms (viruses, fungi, and bacteria) cause a significant reduction in crop yield loss, and perhaps all plant species are affected by them.

Plant parasitic nematodes completed their life cycle partly in the plant tissue or soil and feed on plant cells. The average yield loss of food crops caused by plant-parasitic nematodes has been reported to be about 5% of agriculture production valued USD \$125-157 billion worldwide (Chitwood 2003; Abad et al.2008). Although, Koenning et al (1999) estimated that this amount of yield loss and agriculture food can reach up to 20%. Agriculture production is an important part of Turkish economy. Chickpea (*Cicer arietinum* L.) is an ancient crop that has been grown in India, in the Middle East and parts of Africa for many years. Also, it is an important crop and is a widely cultivated food legume in Turkey. It may have been grown in Turkey for nearly 7,400 years and is the second most important pulse crops grown after beans (FAOSTAT 2011 data for 2009). It is an important protein source for any part of the world (Faostat 2007), and in many countries, particularly India, it also accounts for 65% of world production of chickpea (Singh et al. 2008). More species of plant parasitic nematodes were found in the root and rhizosphere of the chickpea in the wider growing regions of the world. Sasser & Freckman (1987) estimated that 13.7% of worldwide yield losses in chickpea by various plant-parasitic nematodes can make chickpea plants susceptible to disease and other stress (Sikora et al., 2005). The cool-season food legumes such chickpea is parasitized by many nematode species. The most damaging species include the root lesion (*Pratylenchus* spp.), stem (*Ditylenchus dipsaci*), root-knot (*Meloidogyne* spp.) and cyst nematodes (*Heterodera* spp.).

Plant parasitic nematodes are considered to be major pests of agricultural crops and have a global economic impact of more than \$100 billion every year (Atkinson et al., 1995). In addition, the root lesion nematodes are the greatest threat to chickpea production after root-knot nematodes (Barker & Noe., 1987; Jatala & Bridge., 1990). The root lesion nematode, *P. thornei*, and *P. neglectus* were a big problem in the Australian grain region (Riley & Wouts., 2001; Riely & Kelly., 2002; Hollaway et al., 2008; Thompson et al., 2010). Among the root lesion nematodes, *P. thornei* and *P. neglectus* have a worldwide distribution (Nicol et al., 2004), and, both species of nematodes are feeding and reproducing inside the plant root tissue. The damaged chickpea production includes affected plants showing stunting leaf chlorosis, root necrosis by feeding root and reduction of root growth and yield losses greater than 50% can occur in some chickpea variety as a result of this destruction. (Sasser JN & Freckman DW, 1987; Castilo PN & Jimenez RM 1998; Castilo P & Vovlas N., 2007). Also, nematodes may collaborate with other soil-borne pathogens increasing the disease severity. In addition, they may reduce the root nodulation of legumes by rhizobia, limiting nitrogen fixation in cropping systems, and reduce the nutritional quality in the chickpea plant. For controlling nematodes, correct diagnosis of the nematode species and rotation with non-host crops and use of resistant crop cultivars are extremely important. In other words, use of tolerant cultivar can grow and yield well in an extremely infested region and the use of resistant cultivar can deter nematode multiplication (Trudegil.1992).

Earlier research with a limited number of wild chickpea accessions, *C. reticulatum* and *C. echinospermum* showed the potential for obtaining effective resistance genes to root lesion nematodes for use in chickpea breeding program (Thompson et al., 2001). A study (Barker & Noe., 1987; Jatala & Bridge., 1990) has shown that *D. dipsaci* is one of greatest threats to chickpea production after root lesion, root-knot, and *Heterodera ciceri* nematodes. The main aim of the current study is to establish a sound methodology for assessing wild chickpea accessions and a screening method for chickpea varieties against *P. thornei*, *P.*

neglectus and *D. dipsaci* nematodes, to find the source of resistance by different inoculation methods, experiment tube size, growth media, culture technique and time of assessment. Large number of plant parasitic nematodes such as root lesion nematodes infest and attack the underground parts of plants, while a few of this nematode species such as stem and bulb nematode (*D. dipsaci*) develop and the feed on the aboveground plant parts (seeds, stems, leaves).

The nematode genus such as *Ditylenchus* Filipjev, 1936 (*Aunguinidea*, *Tylenchidea*) is widely distributed around the world and widespread group of migratory endoparasitic nematodes. Between these genera, the *D. dipsaci* (stem and bulb nematode) is the most economically important and serious parasitic nematode of the many plant species, in most climates of the world. Susceptible plants include the onion, alfalfa, potato, garlic, oat clover and various legumes crops, such as chickpea. These species of nematode pests live inside the plant tissue usually in the aboveground parts (leaves, stems, flowers) (Subbotin et al., 2005).

This study was undertaken with an understanding of factors that impact yield loss caused by lesion nematodes (*P. thornei*, *P. neglectus*) and *D. dipsaci*.

The objectives of this study were:

- ✓ To identify whether these nematodes cause yield loss in chickpea (optimization).
- ✓ To find the best initial nematode density and harvest time in this research.
- ✓ To evaluate the population dynamics of RLN (*P. thornei*, *P. neglectus*) and *D. dipsaci* nematodes on legumes (chickpea).
- ✓ To determine chickpea varieties with resistance to nematodes.
- ✓ To evaluate the variation between the geographical populations of *P. thornei*, *P. neglectus* and *D. dipsaci* in Turkey.
- ✓ To research sampling strategies for nematodes in field experiments.



2. LITERATURE REVIEW

To evaluate the effect of plant-parasitic nematode species within a cropping system, knowledge on the life cycle (survival strategies) and population dynamic must be determined. In other words, this information, mixed with the development of sampling technique and strategy, will support the description of both host range (including susceptible and resistant varieties) and yield loss in a field experiment.

Chickpea (*Cicer arietinum* L.) is the world's third largest production crop and the third most important crop grown over the second largest area worldwide (FAOSTAT 2011).

2.1. Chickpea and plant parasitic nematodes

Chickpea is a major source of protein, up to 25% in many countries, especially in India which accounts for 65% of the world's production of chickpea (Singh et al., 2008). Singh et al., (1994) and Sudupak et al., (2002) indicated that the average chickpea yield in the world is changing due to the impact of many biotic and abiotic limitations.

This plant is the major crop of the Turkey grain belt. Also, grain legumes usually are grown in rotation with other cereal and oilseed crops. Between legumes, the chickpea crop is valuable in farming systems and has the ability to fix nitrogen partially for their own requirements and also to provide nitrogen to the soil for rotated crops. Di vito et al (1992) reported a more than 58% loss in plant grain and biomass yield of chickpea where the population density of root lesion nematodes exceeded 2000/L of soil in a Syrian field.

Castilo et al (1995) observed among all chickpea cultivars tested, certain varieties were susceptible to root lesion nematodes (*P. thornei* and *P. neglectus*), similarly, Greco et al (1988) found this result in some chickpea cultivars in Syria.

Research (Thompson et al., 2000) has shown that responses to nematicides in the seed yield of chickpeas impacted by root lesion nematodes were in the range 25-60%. Chickpea is one of the most important hosts of both *P. thornei* and *P. neglectus* in Australia (Taylor et al., 2000). Fortuner (1977) reported that the chickpea plant was one of the most important hosts for the lesion nematode (*P. thornei*). Table 1 shows the chickpea production calendar TBL and difference of sowing and harvesting time in the world (2017).

Table 1. Global chickpea production calendar

	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec
Australia					←→					←→		
Canada				←→				←→				
Turkey/Syria					←→						←→	
India (rabi)				←→						←→		
India (kharif)						←→				←→		
Pakistan			←→							←→		
Egypt				←→				←→				
EU (spring)		←→						←→				
China (north)		←→							←→			
China (south)			←→						←→			
Sowing	←→											
Harvest			←→									

Pulse Australia-Northern guide 2016 (Pulse aus.com.au)

2.1.1. Morphology of chickpea

Two common types of chickpea are ‘desi’ and ‘Kabuli’. There is a difference between two types as desi has dark coloured seeds, smaller (4-8 mm) and angular, while Kabuli was beige coloured large seeds (7-14 mm). Desi is mostly split or ground and used for flour in India, while Kabuli is mostly used in ground production in Asian countries and the Mediterranean. Van der Maesen (1987) reported that *C. arietinum* is the only planted belonging of the *Cicer* genus that is a member of the Leguminosae family and Cicereae Alef tribe. The plant has

a deep root system. Also, it is a winter grown legume and an annual plant that stands between 20 cm and 1 m tall (Muehlbauer and Tullu, 1997). It looks like a small bush with frond-like leaves and a number of the woody stems from the base. Work (Oplinger et al., 1997) has shown that the plant leaves are closed with glandular hairs that produce acidic exudates, which play a serious role in the tolerance to insect pests. Generally, Chickpea flowers are white, pink, blue or purplish in colour, but sometimes mixed two or three these colours. The pod formation includes one to three seeds of varying sizes, colour, and shape. Kearns (1991) and Cater (1999) showed that the variation of seed characters used in the basis for the classification of kabuli and desi are the two most important and main commercial types of chickpea. Research (Muehlbauer, 1994) has shown that Accession of wild *Cicer* spp. (*C. reticulatum* and *C. echinospermum*) can be used as a valuable source of resistance genes for cultivated legumes (chickpea). Associated wild *Cicer* species have been classified within primary, secondary and tertiary gene pools to show a growing degree of difficulty to make and produce fertile hybrids with this cultivated species.

Therefore, there are eight annual *Cicer* species of which two are in the primary gene pool with the cultigen *C. arietinum*, namely the direct progenitor *C. reticulatum* La Nijinsky, and *C. echinospermum* PH Davis (Sing et al., 2008), and three in the secondary gene pool, namely *C. bijugum* kH Rechinger, *C. pinnatifidum* Jaubert & Spach and *C. judaicum* Boissier (Singh et al 2008; Croser et al.2003). Both *Cicer reticulatum* and *C. echinospermum* are observed in Turkey's south-east region (Berger et al 2003). The Desi chickpea was developed directly from *C. reticulatum*, while the Kabuli is from Desi or *C. reticulatum* (Toker 2009).

2.1.2 Distribution and classification of chickpea

Previously, Chickpea was placed in the Viciaeae tribe of the legume family including Lentil vetch and fava beans. During the vascular anatomy and pollen morphology, the tribe Cicereae was separated from other member families of the

Vicieae (Kukicha 1977). Until now, Van der Maesen (1987) and Muehlbauer (1993) reported that there have been 43 species for cicer, including 33 perennials, 9 annuals (including *C. arietinum*) and 1 unspecified.

The cultivated species of chickpea is called different names in different countries, for example, it is called chickpea in English, Bengal gram (Indian), garbanzo (Latin America), hamaz (Arab world), shimbira (Ethiopia) and nohut (Turkey) (Muehlbauer and Tullu 1997). Van der Maesen (1987) showed that the chickpea was one of the first grain legumes to be domesticated in the old world. Previously botanists started to follow geographical areas and origins, for example, De Candolle in 1883 began to follow the genesis to an area north of Persia and in the south of the Caucasus. Although, Vavilov (1926) found two primary centers of origins in the Southwest Mediterranean and Asia, and one center of genesis, Ethiopia, based on the genetic and seed analyses that Ladizinsky and Adler (1976) indicated, *C. reticulatum* is the wild progenitor of chickpea and south eastern Turkey is the crops center of origin.

Also, similar studies by Van der Maesen (1987) showed that the south-eastern part of Turkey neighbouring Syria as the possible source of origin of chickpea based on the possibility of the closely related annual species, *C. echinospermum* and *C. reticulatum*. The identification of the close relations between chickpea species in the genetic researching literature was initially completed by Robertson et al., (1997), Ladizinsky and Adler (1976) and Ahmad et al., (1988). The results from the interspecific investigation (Singh and Ocampo, 1997), pattern analysis (Labdi et al., 1996) and chromatin characterization (Galasso et al., 1996) and morphology (Ocampo et al., 1992) between wild relatives and chickpea showed that *C. arietinum* developed from *C. reticulatum*. Also, a similar study using DNA based molecular markers to directly assess genetic variation confirmed that *C. reticulatum* was the progenitor of the cultivated chickpea. Disease in chickpea plants is caused by different pathogenic microorganisms including bacteria, fungi, and plant parasitic nematodes. Some of the most

important plant-parasitic nematodes for chickpea plants are listed in Table 2. To reduce or avoid problems with plant-parasitic nematodes and other diseases legumes crops, such as pea and chickpea, it has been recommended to grow crops in different fields and should be planted with crop rotation (Agriculture and Agri-food Canada, 2013).



Table 2. Some of the most important plant parasitic nematodes for chickpea in the world.

Nematode species	Country	Nematode species	Country
Root-knot nematodes		<i>Helicotylenchus</i> spp.	
<i>Meloidogyne</i> spp.		<i>H. abunaamai</i>	India.
<i>M. arenaria</i>	Ghana, India, North Africa.	<i>H. digonicus</i>	Pakistan.
<i>M. artielli</i>	North Africa, Syria.	<i>H. dihystra</i>	India.
<i>M. incognita</i>	Brazil, Egypt, India, Pakistan	<i>H. indicus</i>	India.
<i>M. javanica</i>	Brazil, Ethiopi, India.	<i>H. retusus</i>	India.
<i>P. alkani</i>	Syria.	<i>Merlinius brevidens</i>	India, Spain.
<i>P. erzurumensis</i>	Turkey.	<i>M. microdorus</i>	Spain.
<i>P. leiocauda</i>	Turkey.	<i>Paratrophurus loofi</i>	Spain.
<i>P. ritleri.</i>	Algeria, Tunesia.	<i>Scutellonema brachyurum</i>	India.
<i>Pratylenchus</i> spp.		<i>Trophurus imperialis</i>	Spain.
<i>P. aleni</i>	Turkey	<i>Tylenchorhynchus</i> spp.	
<i>P. brachyurus</i>	Brazil, Turkey.	<i>T. annulatus</i>	India.
<i>P. coffeae</i>	India.	<i>T. brassicae</i>	India.
<i>P. mediterraneus</i>	Syria, Turkey.	<i>T. clarus</i>	Spain.
<i>P. mulchandi</i>	India.	<i>T. elegans</i>	India.
<i>P. neglectus</i>	Australia, North Africa, Spain, USA.	<i>T. mashhoodi</i>	India.
<i>P. penetrans</i>	North Africa, Spain, Turkey.	<i>T. nudus</i>	India.
<i>P. thornei</i>	Australia, India, Mexico, North Africa, Turkey.	<i>Mesocriconema</i> spp.	
<i>Heterodera</i> spp.		<i>M. curvatum</i>	Spain.
<i>H. cajani</i>	India.	<i>M. ornata</i>	India.
<i>H. ciceri</i>	Syria, Turkey.	<i>M. spaherocephalum</i>	Spain.
<i>H. goettingiana</i>	North Aferica.	<i>Paratylenchus</i> spp.	
<i>H. swarupi</i>	India.	<i>P. microdorus</i>	Spain.
Reniform nematodes		<i>P. sheri</i>	Spain.
<i>Rotylenchulus</i> spp.		<i>Xiphinema</i> spp.	
<i>R. reniformis</i>	India.	<i>X. pachtaicum</i>	Turkey.
<i>R. macrosoma</i>	Syria.	<i>X. index</i>	Turkey.
Stem and bulb nematodes		<i>X. italiae</i>	Turkey.
<i>Ditylenchus dipsaci</i>	Australia.	<i>X. basiri</i>	India.

Data from several sources (Ali, S. S., and Sharma, S. B. 2003; Castillo et al., 1996; Divito et al., 1994; Sharma et al., 1998 and 1992; Singh et al., 1989).

2.2. *Pratylenchus thornei* and *Pratylenchus neglectus*

Root lesion nematodes or *Pratylenchus* spp., are observed worldwide and infest a wide range of plant species. *Pratylenchus* and the genus of this nematode species were first reported by Filipjev (1936). The special species of this genus are migratory endoparasites and vermiform between 0.3 mm and 0.8 mm in length with similar morphological characteristics. Plant parasitic nematodes cause significant damage to legumes (such as the chickpea) in various Mediterranean countries (Greco, 1985; Greco and Di Vito, 1988; Sikora and Greco, 1990; Greco et al., 1992; Di Vita et al., 1994).

Research (Di Vito and Greco, 1986) has shown that *Heterodera goettingiana* Liebscher, causes serious damage to pea (*Pisum Sativum* L.) and the faba bean (*Vicia faba* L.). Greco et al., (1984, 1992) and Di Vita et al., (1994), reported that the most common nematodes were *P. thornei* and *P. neglectus* in chickpea and lentil fields in Syria and North Africa. Research (Barker & Noe., 1987; Jatala & Bridge., 1990) has shown that the *D. dipsaci* nematode is one of the greatest threats to chickpea production after root lesions, root-knots, and *Heterodera ciceri* nematodes. This nematode is damaging to chickpeas (Di Vito and Greco, 1988; Greco et al., 1988). The legume's nematodes in Turkey are of crucial significance because Turkey ranks second in the world in chickpea production and the first in lentil production in the world.

Both species of root lesion nematodes, *P. thornei* and *P. neglectus* have a similar host range and are observed in the dry land region in South Australia. *P. thornei* and *P. neglectus* were first reported in 1950 and 1956. Most plant diseases and yield losses caused by other pathogens were confused with disease symptoms and yield losses caused by these nematodes as it was not clearly identified until 1990.

2.2.1 Life Cycle

Root lesion nematodes are migratory endoparasites eel-worms 0.5 mm-0.7 mm long, unlike sedentary nematodes, these nematodes do not have a fixed feeding site and move between fertile soils and reproduce in the root cortex. They prefer to attack the root 3 mm to 13 mm behind the root tip (Castillo & Vovlas 2007). Zunke (1990) discovered that when these nematodes enter the root, they move between cells and starting to feed by penetrating the cell with a stylet which finally leads to cell destruction in the plant.

Dropkin (1980) showed that due to activities of the root lesion nematodes observed, there was a reduced uptake of nutrients and water by the damaged roots. In *P. thornei* and *P. neglectus*, males are not required for reproduction due to parthenogenesis and can also increase without parthenogenesis but reproduction with males rarely occur. (Nicol., 1996; Taheri., 1996). Eggs are placed inside the root, and the first molt occurs inside the egg. After hatching, later molts occur by the second and forth juvenile stages to the adult. The life cycle is completed after 45–65 days, however the range of the life cycle of these nematodes depends on the environmental conditions, such as temperature, food source, and differences between *Pratylenchus* species. This species has been observed to complete three to six generations inside plants during a single growing season.

Work siyanand et al. (1982) has shown that females of *P. thornei* completed a life cycle in 25-29 days at 30-32°C and determined that feeding them from inside the root was important for growth and development. In southern Turkey's dryland cropping region, the main crop, chickpea (*C. arietinum*) is usually between March and July. Meagher (1970) indicated that *P. neglectus* can live in the dry soil for fifteen months. During the summer period, the population density of *P. thornei* and *P. neglectus* have been indicated in high numbers both in the root and soil.

2.2.3 Symptoms

Root lesion nematodes attack the cortex of the plant root and move between cells to feed. These behaviours of root lesion nematodes caused damage to the cortical cells. Castilo and Vovals (2007) indicated that the high population density of root lesion nematodes, with their destructive effects on root function, cause reduced plant biomass, leaf chlorosis, loss of yield in a harvestable product and a reduction in root hairs. *P. neglectus* is an example of a reduction in root hairs of plants in South Australia. Some root hairs or roots may be suitable and vulnerable to attack than others from plant parasitic nematodes such as cereal and legumes. Also, Zunke (1990) indicated that the effect of lesions ranged in colour, changing from orange to dark brown in the plant as infestation by the nematode increases. The research by Baxter and Blake (1967) showed that the population density of *P. thornei* was established in the region of heavy contamination within wheat roots. Sometimes, the symptoms on the roots of field crops are usually not visible until 6-8 weeks after sowing, as this period of time is needed for nematodes to multiply and for roots to contain adequate levels to produce necrosis. Symptoms above ground are caused by Lesion nematodes and are not specific and usually impact the same area of plant growth. Research (Van Gundy et al., 1974; Farsi et al., 1995) has shown that all of the plants affected by lesion nematodes were stunted, with a reduced number of shoots and yellowing of leaves. Both species of root lesion nematodes (*P. thornei* and *P. neglectus*) are feeding and reproducing in the root of different species of plant families.

2.2.4 Interaction with other pathogens and identification of nematodes pathogenic to chickpea

Wallace (1983) reported that there is a complex system between beneficial organisms and pathogens affecting plant growth in the field. As it is possibly difficult to separate the particular effects of these organisms or identify that the effects are more or less than the amount of the effects of the organisms, therefore it

is possible to show an incorrect interaction. The correct diagnosis of plant-parasitic nematodes that are infesting crops is a necessity for organizing efficient management strategies. A study by Perry and Moens, (2006) has shown that effective management strategies are necessary for chickpea, where different species of root lesion nematodes (*P. thornei* and *P. neglectus*) are critical pathogens. The determination of these species of plant-parasitic nematodes is usually based on morphology and morphometric of adult females or several DNA based analyses of nematodes.

Plant parasitic nematodes such as root lesions and *D. dipsaci* (stem and bulb) nematodes that attack chickpea can interact with soil-borne microorganisms (including beneficial and pathogenic microorganisms) as they inhabit a similar ecological in the chickpea rhizosphere. These plant-parasitic nematodes interactions with other pathogens are very important biologically and phytopathologically for chickpea production. Research (Nour et al., 1995) has shown that free nitrogen fixation by chickpea roots can enable the cultivation of crops in many nitrogen-poor soils with *Sinorhizobium* and *Mesorhizobium* rhizobia. Soussi et al. (1999) indicated that the yield performance of chickpea depends on the associated plant genotype and rhizobia that together affect the symbiotic potential and symbiosis reaction to abiotic and biotic stresses. Hussey (1987) showed that plant parasitic nematodes by associated rhizobia roots and nitrogen fixation can affect symbiotic nodulation on legumes' roots (such as the chickpea).

In a similar laboratory study confirmed that plant parasitic nematodes such as *Meloidogyne incognita*, contaminate more than 25% of nodules induced by *Mesorhizobium* in chickpeas (Vovlas et al., 1998), where the nematode infection nodules established permanent feeding sites in the vascular bundles of the nodules. Research has shown that nematode infection of nodules impacted the legumes' roots and caused loss in number of functional nodules per root system on different chickpea cultivars. Similarly, another study confirmed that nematode infection

nodules by root lesion nematodes (*P. thornei*) caused a reduction in the number of nodules root system on chickpea (Darekar et al., 1987; Tiyyagi et al., 1992).

Plant parasitic nematodes play an important role in the improvement of other chickpea diseases produced with soil-borne pathogens. The interaction between nematodes and other pathogens such as fungi may be different depend upon the plant genotype, lines, and cultivars in the study of disease interactions. Indeed, a number of universal experiments have been reported regarding the interactions between *Pratylenchus* species (*P. thornei* and *P. neglectus*) and other pathogenic fungi such as *F. oxysporum* and *F. sp. ciceris*, that plays a significant role to improve the Fusarium wilt disease in the chickpea (Bhatt et al., 1997; Uma et al., 1997; Castillo et al., 1998-2003). Another study (Mani et al., 1987; Krishna Rao et al., 1996; Uma Maheshwari et al., 1997) has shown that the interaction of plant-parasitic nematodes with other pathogens play a significant role in disrupting resistance in chickpea plant against other diseases and lead to destroy the resistance in chickpea plant against other plant pathogens. Evans and Haydok (1993) showed that the interaction of nematodes with other pathogens caused doors to open and enable other diseases to enter plants root by feeding root lesion nematodes in roots, which led to observe symptoms such as yield loss and performance in the chickpea.

2.2.5 Host range and economic importance

The *Pratylenchus* genus nematodes *P. thornei* (Sher & Allen, 1953) and *P. neglectus* (Rensch, 1924; Filipjev Schuurman & Stekhoven, 1994) have a wide distribution in 82% of the fields in many regions and affect many agricultural crops in the world (Tanha Maafi et al., 2009). Stirling et al. (1992) reported that the genus of *Pratylenchus* (*P. thornei* and *P. neglectus*) are very important plant pathogens and is the most widely dispersed pathogen throughout cropping rotation systems in the world.

After *Meloidogyne* (root-knot nematodes) and *Heterodera* (cyst nematodes), the *Pratylenchus* genus is the most damaging nematode to crops in Australia. Also,

both the *P. neglectus* and *P. thornei* have been reported in a large host range of crops and geographic distribution in many countries in the world. The determination of plant-parasitic nematodes damage is not easy because it may be affected by other pathogens or disease, environmental factors and degree of tolerance in the host plant. A similar result showed that plant-parasitic nematodes caused more than \$AUS300 million of economic damage in fertile soil crops in Australia. However, the root lesion nematodes played the main role to cause this economic damage in many field crops. A similar result indicated that *P. thornei* and *P. neglectus* caused a yield loss of approximately \$AUSD 44.3 million and \$AUS80.5 million of economic damage in field crops in Australia (Brennan and Murray 1998).

2.3. *Pratylenchus thornei*

Pratylenchus thornei Sher and Allen is one of the most important plant-parasitic nematodes causing yield losses of up to 40% in cereals and legumes in the drylands and the major cropping areas of south-east Australia (Thompson et al., 1995; Vanstone., 1998). Yield loss caused by root lesion nematodes (*P. thornei*) was largely observed in many fields and countries. Research (Thompson et al., 1995) has indicated losses of 42% for an intolerant wheat variety in Queensland. Similarly, studies in northern New South Wales demonstrated that *P. thornei* is a large factor limiting the yield of cereal and legumes in many regions, where yield losses of up to 80% have been indicated. The use of nematicide treatment for *P. thornei* was beneficial for yield increases where an increase of 37% was indicated compared with only the application of high rates of nitrogen, zinc and phosphorus which caused minimal benefits (Doyle et al., 1987).

Research (Vanstone et al., 1998; Nicol et al., 1999) shows that in different studies regarding the interaction of nematodes, the numbers of yields in the susceptible and resistant variety of *P. thornei* caused yield losses of 20% and 30% on legumes (chickpea) and cereal crops, respectively, in South Australia. Divito et

al. (1992) reported that *P. thornei* is a major factor limiting the yield of chickpea in the growing areas of Syria. Similarly, studies in India confirmed that *P. thornei* has been identified as a major pathogen in these areas and has become clearly the most damaging impact where intolerant legumes and cereals are grown in drier areas (Haseeb and Shukla 1995).

2.4. *Pratylenchus neglectus*

The root lesion nematode, *P. neglectus* (Rensch) Filipjev Schoomans Stekhoven is a migratory endoparasite of crops in different climatic regions of the world. Chickpea, wheat, and canola are good hosts for this nematode, while barley, oat, and vetch are moderate hosts. While, field faba bean, pea and triticle are poor hosts for this nematode. These nematodes caused yield loss in alfalfa (Griffin, 1991; Griffin and Gray, 1990), barley (Ferris et al., 1994; Umesh and Ferris, 1992) and potato (Olthof, 1990). Chickpea is a major host of *P. neglectus* in the large cropping and dry areas of south-eastern Australia. Work (Taylor et al., 1999; Vanstone et al., 1998) has shown that this caused yield losses of about 20%, although, both the *Pratylenchus* species of *P. neglectus* and *P. thornei* have a similar effect and host ranges in southeastern Australia.

2.5. *Ditylenchus* genus

Ditylenchus filipjev, 1936 is one of the most important plant parasitic nematodes that affected most agriculture crops (Fortuner, 1982). Various types of pests can infect the plant. Plant-parasitic nematodes are one of the most important pests that attack agricultural plants and hundreds of nematodes can attack crops. In fact, many can parasitize just one plant variety, which means that each nematode has a specific host where it causes yield losses. Sothey (1978) indicated that plant-parasitic nematodes feed on crops using a special organ (stylet) to infect the plants. Plant parasitic nematodes generally feed on different organs of the plant, especially on roots and other subterranean plant structure such as rhizomes in legumes crops.

Also, Sasser (1989) indicated that the feeding habits of these plant-parasitic nematodes depend on their innate behaviour, with some being ectoparasites living in and out of plant tissue (sedentary or migratory), and others being endoparasites (sedentary or migratory) entering plant tissues.

One of the most important species of plant-parasitic nematodes is *Ditylenchus filipjev*, 1936 and it infects almost every agriculture crop (Fortuner., 1982). Siddigi (2000) reported that the *Ditylenchus* genus comprises more than 60 to 80 genus and spread quickly with a wide variety of hosts in the world. Plant parasitic nematodes cause major damage to legumes (chickpea) in different Mediterranean countries (Greco, 1985; Greco and Di vita, 1988; Sikora and Greco, 1990; Greco et al., 1992; Di Vito et al., 1994). Research (Di Vito and Greco, 1986) has shown that *H. goettingiana* Liebscher causes serious damage to pea (*Pisum sativum* L.) and the faba bean (*Vicia faba* L.). Greco et al., (1984, 1992) and Di Vito et al., (1994) all reported that the most common nematodes were *P. thornei* and *P. neglectus* in chickpea and lentil fields in Syria and North Africa. These nematodes are damaging growth in chickpea fields (Di Vito and Greco, 1988; Greco et al., 1988).

The plant parasitic of legumes is very important in Turkey because it is a country which ranks second in chickpea production and the first in lentil production in the world. Work (M. Divito et al., 1992) has shown that root lesion nematodes were the most common of the several plant-parasitic nematodes associated with chickpea and lentil in Turkey during spring 1991 and summer 1992. Also, *P. thornei*, *P. penetrans* and *P. mediterraneus* were more observed in the Chickpea samples. Also, among other *Heterodera* spp., *H. ciceri* was detected in twelve fields of chickpea and two of lentil however severe damage was observed only on chickpea. Nematode infected Chickpea plants were stunted and produce fewer flowers, pods with small or no seeds. It was very damaging to Chickpea. Also, between root knot nematodes *M. artiellia* was indicated in one sample collected at Denizli. The literature (Barker & Noe, 1987; Jatala & Bridge, 1990)

has shown that *D. dipsaci* was one of the greatest threats for chickpea production after root lesion, root-knot and *H. ciceri* nematodes that can cause yield loss in chickpea growing areas.

2.5.1. The most important species of the *Ditylenchus* genus

The *Ditylenchus* species has significant diversity in the conditions of feeding habits and has successfully adapted to many of agricultural crops in the world, predominately this genus of parasitic nematodes live in the root and shoots, and rhizomes. Many species of plant-parasitic nematodes live in different part of crops, such as shoots, roots, and rhizomes. Research (Prowright et al., 2002; Zhang et al., 2014) has shown that only six genera of *Ditylenchus* have important economic impacts and are the predominant pests of agriculture crops. These are *D. dipsaci* (Kühn) Filipjev (stem and bulb nematode), *D. destructure* Thorn (potato nematode), *D. angustus* (Butler) Filipjev (rice stem nematode), *D. gigas* (broad bean stem nematode), *D. africanus* (peanut pod nematode) and *D. arachis*. Chizhov et al. (2010) and Vovlas et al. (2015) reported that other species of *Ditylenchus* are living in the soil such as *D. oncogenus* and *D. Weischeri*, but they cause no damage to the host plant.

2.5.2. Stem and bulb nematodes (*Ditylenchus dipsaci*)

D. dipsaci (Kühn) Filipjev 1936 was indicated by Schwartz for the first time on an easel, *Dipsacus fullonum*, in Germany in 1855, after two years Kühn officially showed the nematode in 1936, Filipjev described this (Kühn 1857; Brzeski and Sturhan 1991). The stem and bulb nematode is one of the most economically impacting plant-parasitic nematodes in the farming system worldwide. Work (Sasser., 1989; Brzeksi., 1991) has shown that *D. dipsaci* be formed and developed in humid climates such as Southern and Northern Asia, Africa, Europe and South America. Plowright et al. (2002) indicated that the population density and reproduction of the *D. dipsaci* nematode was increasing in

moist and rainy conditions. This nematode is listed as an A2 in between important European countries (EPPO 2015). Also, *D. dipsaci* nematode is capable of wide distribution, which damages plant materials and seeds of host crops, moves the location of soil fields, irrigation water, infected agriculture equipment. Phytosanitary certification systems were used in many countries as a special technique and method to prevent spreading of this nematode.

2.5.3. Host of *Ditylenchus dipsaci*

The capacity of a nematode species to improve and reproduce on a host plant is defined as the suitability of plant species for a parasitic nematode. About 450 to 500 different plant species such as agricultural, nursery and horticultural are widely ranging hosts available to *D. dipsaci*. Lewis (1987) reported that the suitability of plant species (host crops) determined the multiplication rate and the population density of nematodes. The stem and bulb nematode is a migratory endoparasite with fourth stage juveniles being the most infectious stage. These nematodes are seed and soil borne nematode and attacks the below and above ground parts of host plants and are able to damage the upper parts of the legumes plant such as pods and seeds. Similar studies indicated that infested pods and seeds are significant effects of spreading this nematode pest (Hooper (1971). In addition, (Hussey and Krusberg) indicated that this nematode caused yield losses in legumes crops, whereas Krusberg and Chitwood (1977) indicated that a population density of *D. dipsaci* can make a gall formation in seedlings of a resistant variety of legumes.

2.5.4. Crop loss caused by *Ditylenchus dipsaci*

D. dipsaci occurs with serious effects on agriculture crops especially in warm temperature rainy areas. The nematode damage is mostly increased during humid, cool weather in fields watered with sprinkler irrigation systems and also in hazy areas. Major host crops for these nematodes include onion, alfalfa, oats, rye,

and legumes such as, pea, field beans, and chickpea. Research (Sturhan and Brzeski, 1991; Plowright et al., 2002) has shown that even a low population density of *D. dipsaci* can create high densities that result in serious loss to the cultivated host. Surveys undertaken in Turkey, during spring of 1991 and summer of 1992, revealed that *D. dipsaci* was present in two samples of lentil and chickpea (M. Divito et al., 1992)

Research (Bridge and Starr 2007) has indicated that the threshold damage level of *D. dipsaci* per gram of soil is a population density of ten or more nematodes per 500 cm³ soil (Seinhorst, 1959). Nicklev (1991) found a crop loss of 60% to 80% in soils heavily infested by *D. dipsaci* nematode. Similar studies in England confirmed that a yield loss of up to 37% was due to an infestation of this nematode species on oat crops (Whitehead et al., 1983). The interaction of this nematode upon legume crops is indicated by *D. dipsaci* causing yield loss on legumes crops such as pea, chickpea, and lentil during humid seasons (Greco and Di Vito, 1994; Bridge and Starr, 2007). Similar studies in the UK observed a yield loss of up to 70% in Vicia beans (Biddle and Cattlin). Also, research (Griffin, 1990 and Hillnhutter et al., 2011) indicated an interaction of *D. dipsaci* with other pathogens such as fungi and bacteria.

2.5.5. Disease symptoms caused by *Ditylenchus dipsaci*

The symptoms of nematode damage on various crops differ depending on the infested plant species. Usually, *D. dipsaci* creates swelling and distortion on leaves and stems of different susceptible crops where parenchyma cells are destroyed (Sturhan and Brezeski). Damage can be seen on legume crops including, chickpea, peas or faba bean, where *D. dipsaci* damage appears as deformation and swelling of stems and leaves. Seeds effected by this nematode are small and deformed with freckle-like spots (Bridge and Starr 2007; CABI 2015). The stunting and discoloration of plants can be observed due to *D. dipsaci* damage, where finally, the plants turn yellow and die.

2.5.6. Biology and survival of *Ditylenchus dipsaci*

D. dipsaci reproduces sexually after four moults of developmental stages (Hooper, 1972). The infective juvenile stage of *D. dipsaci* is the J4. Normally the life cycle of *D. dipsaci* takes between 19 and 23 days at 15°C, where a mature nematode live for 45 to 73 days, females are capable of producing 200 to 500 eggs (Yuksel, 1960). Also, the reproduction of *D. dipsaci* is influenced by some factors including the growth rate of host crops (Blake 1962), and also ecological conditions such as moisture and temperature.

Temperature can influence the life cycle and development of any plant parasitic nematodes. The greater population density of nematodes can be observed on the surface of parts and seeds of highly infested plants (Hooper, 1972). Wallace (1961) indicated that the temperature optimal for *D. dipsaci* activity and reproduction was between 15 and 20°C, although, the growth of host plant is very important for reproduction of *D. dipsaci* Blake (1962)

2.6. Nematode management strategies

Plant-parasitic nematodes management include physical control, cultural control, biological, and chemical controls. The population density of plant-parasitic nematodes may be reduced by one or multiple control method. Nicol (2002) observed the choice of a suitable method to manage the population density of nematodes must be selected based on nematode species, the interaction of nematode with host plants and other diseases and nematode biology. Hughes et al. (2013) indicated that no beneficial control method is available to prevent the spread of the plant parasitic nematodes and minimize the nematode damage.

Although, there are different methods to control the nematode damage including cultural control (crop rotation, antagonistic and cover crops), physical control (soil solarization and hot water treatment), the use of plants resistant to plant parasitic nematodes is economically important. The use of uninfected and certified seeds is one strategy that is efficient against nematodes. Similar studies in

Canada stated that the extensive distribution of *D. dipsaci* was due to using infested garlic bulbs by a producer. The other cultural methods to control plant parasitic nematodes is crop rotation, in other words using non-host crops to reduce the population density of the growing soil host plants. Crop rotation with non-host plants for 3 to 4 years usually efficient to decrease soil population of the nematode (Lorbeer et al. 1997).

Similar studies in California stated that producer uses a 4-year crop rotation of non-host plants between garlic growing to control *D. dipsaci* (Roberts and Matthews 1995). The other methods to control the population of nematodes in agricultural crops is used from an antagonistic plant and cover crops that are helpful for decreasing population density of nematodes in growing soil field, protect soil from erosion and development soil texture (Nusbaum and Ferris, 1973; Trivedi and Barker, 1986). Also, Tando et al. (1989) observed that extracted natural nematicidal from some cover crops sometimes cause a reduction in nematode populations in the soil. Work (Chen et al. 2004) has shown that mulching the soil with cover crops such as *Artemisia dracunculus* can be used to manage plant-parasitic nematodes and is effective against nematodes (*D. dipsaci*).

Physical control including soil solarization and hot water treatment can be used to manage population plant-parasitic nematodes. Sometimes used as a non-chemical alternative such as soil solarization is effective against to nematodes. Normally growers can use this method against plant-parasitic nematode by increasing soil temperature to 40 to 50°C which is deadly for nematodes in a warm climate region (Katan, 1987; Whitehead, 1998). Similar research in Italy and Israel stated that the effect of soil solarization reduced the population density of the nematodes in soil (Siti et al. 1982; Greco et al. 1985). The other physical control that can be used to kill nematodes is a hot water treatment. It is usually with different mixes of time and temperature, for example, a hot water treatment of 43 to 44°C for 1 h to sterilize seeds or bulbs (Gratwick and Southey, 1972).

2.6.1. Plant resistance to nematodes

Some agricultural plants have specific resistance genes that protect them against damage from plant-parasitic nematodes. Usually, resistance is defined as the ability of a plant to decrease or prevent the population density and reproduction of nematodes (Trudgill et al., 1998). Research (Trudgill 1991; Cook and Star 2006) has showed that use of the plant resistance method is one of the most important and economical application to control and manage plant-parasitic nematodes. Sturhan and Brzeski (1991) have found certain varieties of plants such as pea, field bean, and chickpea against plant-parasitic nematodes with different levels of sensitivity. Also, Brown (1998) indicated that growing resistant cultivars and crops can reduce the damage caused by initial population densities of plant-parasitic nematodes on agricultural systems.

Plant resistant cultivars to control of nematodes are useful within crops rotation (non-host plant) method. Also, Cook and Evans (1987) reported that cultivars may be completely resistant (allowing no nematode reproduction) or partial resistant (allowing intermediary levels of nematode reproduction). For migratory endoparasites such as *P. thornei*, *P. neglectus* and *D. dipsaci* partial resistance occurs more commonly. There is an example of complete resistance to *Globodera* spp. in potato (Fransco and Evans, 1987) and resistance to *Meloidogyne* spp. in *Prunus* root stocks (Fernandez et al., 1994). An example of partial resistance was reported with *Rotylenchus reniformis* on soybean and *P. penetrans* on alfalfa (Robbins et al., 1994; Thies et al 1994).

Although, chemical controls of nematode can be used to manage and control plant parasitic nematodes, it is very expensive and risks human and animal health as pollute the atmosphere. Also, chemical controls are not working in most soil as 99% of the nematodes must be killed in the soil, or, living nematodes in plant or soil can start to reproduce on host or non- host plant (Whitehead 1998; Sturhan and Brzeski 1991).

Many biological control factors such as nematophagous bacteria and fungi, recommended reducing population density of plant-parasitic nematodes (Sikora 1992; Stirling 2005). Many accessions of varieties and lines of the plant must be usually evaluated to select a resistant cultivar to plant parasitic nematodes. Nelson et al. (1984) observed that the shoot and root weights were increased and the number of nematodes reduced in alfalfa when the selected cultivar resisted the root lesion nematodes. In order to select for resistance in chickpea plant, a large accession of wild *Cicer* spp. must be evaluated. Similar studies observed no resistance to root lesion nematodes (*P. thornei*) in 97 chickpea lines, showing that a large number of cultivars with a wider range of genotypes may be needed to select and identify resistance of this crop (Greco et al., 1988).

The accessions in wild *Cicer* spp. and pasture species resistant to root lesion nematodes (*P. thornei* and *P. neglectus*) and stem and bulb nematode (*D. dipsaci*) is discussed in this project. Also, changes in ecological conditions may change the resistance cultivars and stability of plant resistance. For example, the literature (Jattala and Russel., 1972) has shown crops such as bean, tomato, and potato to be more susceptible to *Meloidogyne* species at a higher temperature. Moreover, interaction between nematode species can be affected by a plant's resistance. For example, Jattala and Russel (1972) showed that resistance to stem nematode (*D. dipsaci*) was destroyed with interaction other nematodes (*Heterodera avena*) in the oat cultivars.

2.6.2. Tolerance

The ability of the plant to yield well despite a high infestation by a numbers of nematodes is defined as tolerance and play an important role in yield loss (Jones 1956). The initial population density of nematodes, host crops, and an environmental condition can be affected by plant tolerance. Also, it is possible that there is no interaction between tolerance, and resistance may be independent of tolerance.

A low initial population density of nematodes may cause damage in resistant and intolerant host crops. Similar studies in South Australia showed that tolerance and resistance often happen together to root lesion nematodes (*P. neglectus*) in wheat (Vanstone et al., 1998). While Thompson et al. (1999) showed that the opposite of this study, varieties have been seen in Queensland, with high tolerance to *P. thornei* but no resistance. Also, varieties of tolerance may be increased due to the number of factors in crops host. The further example, Potter, and Dale (1994) observed varieties of tolerance in strawberry cultivars to *P. penetrans* and recommended tolerance may be increased with some factors including better root growth and sugar production. The other hands, a disadvantage of crops that are tolerant but susceptible.

2.7. Population variability

A change in population density (population variability) has been broadly studied for some species of plant-parasitic nematodes with often enough species characterized for the differences to be called races. For example, studies (Hartman and Sasser 1985) showed four races within *M. incognita* based on different reactions in six hosts. The identification of races appears more easily for sedentary plant-parasitic nematodes with a changing relationship with their host, because of their susceptibility or resistance. However, for migratory nematodes, the identification of races is not often easy or clear and the host range of each is not completely understood.

For example, Sturhan and Brzeski (1991) stated that there are 30 races within *D. dipsaci*, but the host range is not clearly understood. Also, the identification of races does not appear to be clear for the *Pratylenchus* species. Several methods can be used for the identification of population variability; including, reproduction on a different host, evaluation of nematode morphology, and mutation within proteins using allozyme electrophoresis.

2.7.1. Morphology

Classic morphological methods have been used to identify between populations or isolates of plant-parasitic nematodes. Isolation characteristics such as body length, body width and the length of the tail were identified based on morphological measurements and these characteristics were established between isolates. For example, Prasada et al. (1988) found six isolates of the reniform nematodes. Similar studies in North America indicated that 23 populations of *Xiphinema americanum* were identified based on these morphological characters (Cho and Robbins, 1991).

Sometimes, there is a degree of changeability between morphological characters used, and it may be difficult to identify differences between populations, as variation can occur depending on the nutrition of the nematode or host. Singh and Khan (1981) tried to use morphometric measurements to identify variation between *P. thornei* populations and concluded that values such as body length, lip dimension, and body/spear length indicated a major difference even within a single population. Moreover, in regard to the differences in morphological characteristics, the time and level of skill required to identify populations based on morphological studies is quite difficult.

2.7.2. Host range studies

Differentiation in multiplication rates (host range tests) are often used as showing of variability in virulence between populations as this allows an estimation of measurable differences within species. For example, Griffin and Jensen (1997) have indicated differences in reproduction in *P. neglectus* population in wheat grass.

2.8. Spreading, sampling and extraction

The determination of population density of plant-parasitic nematodes and advantages of different sampling and extraction methods for the identification and

estimation of plant-parasitic nematodes in soil crops and the species is important if the evaluation of yield loss or resistance and population dynamics are important subjects within field studies. In this study, the extraction procedures and sampling strategies for root lesion nematodes and *D. dipsaci* in Turkey were assessed prior to the evaluation of yield loss and population dynamics in field experiments. The level of sampling needed depended upon the purpose of the experiment, which may be an estimation, survey, and identification or research. Morris (1960) showed that the best sampling procedures for determining population density of nematodes are essential equipments combined with science and hard work. Also, the distribution of nematodes and the host type play a main role to select the factors to be considered in sampling. In addition, the nematode species can be affected by the sampling methods used. For example, Boag et al (1992) indicated that many subsamples can be taken without mechanical damage with no loss in nematode recovery from some species of nematodes such as cyst nematodes.

Also, Boag and Brown (1985) reported that the minimum size of sampling with no mechanical damage can be used for the large species of plant parasitic nematodes such as *Trichodorus* spp and *Longidorus elangatus* (virus vector nematodes). Also, the sampling equipment used can be affect the results and increase error estimated because samples were taken with different equipment size within different core size (Mc Sorley and Parrado, 1982). The growth stage of planted crops and moisture can also affect the recovery of population density of plant-parasitic nematodes from soil and plants. For example, sedentary nematodes can only be available and present in the soil for the short time before infesting the roots and forming fixed feeding while migratory nematodes can exist in both soil and plant where nematodes surrounded the root in the soil. Because of that, it is important to make a plan for sampling strategies for the purpose of the experiment and nematode species.

2.9. Effect of temperature, time and inoculum density on the reproduction of *Ditylenchus dipsaci* in carrot disk culture

D. dipsaci (Stem and bulb nematode) is a very important plant parasitic nematode, has a very wide range of hosts and causes economic yield losses in many cultural plants in worldwide. Its races are very diverse and found in most temperate areas of the world. *D. dipsaci* is a migratory endoparasite and has the ability to enter into a dormancy stage. Genetic resistance offers one of the best control methods within the integrated pest management strategies for *D. dipsaci*.

The use of carrot culture disks is one of the best ways for reproduction of root lesion nematodes (Huettel, 1985). Different studies have been published about the culture of root lesion nematodes on carrot disks (Chitambar and Raski, 1985; Kaplan and E Davis, 1990; Verdejo and Pinochet, 1992.). Since then, there was no further reported studies about the culture of *D. dipsaci* on carrot disks and no reference reported about factors such as the effect of time, temperature, and inoculum density on nematode reproduction. The aim of the study was to focus on alternative rearing methods using carrot discs as a food source, culturing medium and provide a clearly outlined and visually informative guide. Carrot discs enable the rearing of high numbers of individuals of *D. dipsaci* for timely use in experiments and for screening purposes in under sterile conditions to provide a clean, identical and pure source of inoculum.

The carrot disc method was shown to be suitable for stem and bulb nematode multiplications. Lawn et al. (1984) was looking to find the best methods for culture and increase population density of plant-parasitic nematodes for a long time. But generally, these techniques were not easy and needed a greater understanding of some further related things. While the three most commonly used techniques for culturing migratory endoparasitic nematodes, including alfa callus (Acosta and Malek, 1979; Castillo et al 1994, Draper and Smith, 1981.), root explants (Chitambar and Raski, 1985), and carrot discs (Coolen, 1979) have been published, the main of this study is to use the carrot culture method to increase the

population density of nematodes. Stem and bulb nematode or *D. dipsaci* attacks more than 1200 types of wild and cultivated plants. Also, many agricultural plants are hosts for this species of nematode.

D. dipsaci is a migratory endoparasite nematode and mostly lives in different parts of the plant such as stem, leaves, and flowers. In vitro rearing of *D. dipsaci* nematodes on a carrot disc were usually used for growing nematodes. This method was proven workable for migratory nematodes (*Ditylenchus* spp) to manufacture nematodes for experimental aim, direct studies of nematodes, screening trial and increase nematodes. The greatest advantage of this method was to save time and ensure a quick reproduction of nematode masses.

Most of the plant-parasitic nematode can just be developed in fresh root tissue. In other words, these methods help the production of *D. dipsaci* nematode on sterilized carrot discs in Petri dishes in an incubator greater than root plants under laboratory conditions. Nematodes feeding on two types of plant tissue is necessary for their culture as they can feed as migratory endoparasites and ectoparasites. Sedentary endoparasitic nematodes individuate tissue for reproduction but the opposite is true regarding migratory endoparasites nematodes like *D. dipsaci*. These endoparasites do not require individuate tissues and reproduce easily on undifferentiated tissue like carrot disks and it has been indicated to be suitable tissue for their multiplication. O'Bannon et al. (1968) indicated that this technique provides a basic facility for migratory endoparasites nematodes to be reared on carrot discs in Petri dishes inside an incubator more than plant's root under greenhouse conditions. Moreover, this trial provides a protocol for the use of carrot; to produce and extract stem and bulb nematodes from carrot disk culture.

2.10. Optimization and screening of chickpea genotypes collected from Turkey against the root Lesion aematodes (*P. neglectus*, *P. thornei*) and *D. dipsaci*.

2.10.1. The root lesion nematodes

Chickpea (*Cicer arietinum* L.) 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 nearly 7,400 years ago and is the second important pulse crops sown over after bean (FASOSTAT, 2011). It is also an important protein source for any part of the world (FAOSTAT, 2007). More species of plant parasitic nematodes have been found in the root and rhizosphere of the chickpea in the greatest growing regions in the world. The literature (Sasser & Freckman, 1987) has shown that a 13.7% of worldwide chickpea yield losses by various plant-parasitic nematode feeding. Also, (Sikora et al., 2005) showed nematode attacks to chickpea can be make the plant susceptible to disease and other stress and (Atkinson et al., 1995) showed that they are major pests of agricultural crops and have a global economic effect on crops of more than US\$100 billion every year. Also, research (Barker & Noe., 1987; Jatala & Bridge, 1990) has shown that root lesion nematodes are one of the largest enemies for chickpea production after root-knot nematodes.

The root lesion nematode (*P. thornei* and *P. neglectus*) are a big problem in the Australian grain region (Riley & Wouts., 2001; Riely & Kelly., 2002; Hollaway et al., 2008; Thompson et al., 2010). Nicol et al (2004) reported that the root lesion nematodes of *P. thornei* and *P. neglectus* have a worldwide distribution with both species feeding and reproducing inside plant roots tissue. The damaged chickpea production includes affected plants showing stunting leaf chlorosis, root necrosis by feeding root and reduction of root growth and yield losses greater than 50% can occur in some chickpea variety as a result of this destruction. (Sasser JN & Freckman DW., 1987; Castilo PN & Jimenez RM 1998; Castilo P & VovlasN., 2007).

For nematode control, correct diagnosis of the nematode species and prepare-rotation with non-host crops and use of resistant crop cultivars are

extremely important. In other words, the use of tolerant cultivar that can grow and yield well in extremely infested regions and the use of resistant cultivar that retard nematode multiplication creates the basis for a controlled experiment (Trudegil, 1992). Earlier research with a limited number of wild chickpea accessions, *C. reticulatum* and *C. echinospermum* showed the potential for obtaining effective resistance genes to root lesion nematodes for use in chickpea breeding program (Thompson et al., 2001).

2.10.2. *Ditylenchus dipsaci*

Optimization of chickpea genotypes collected from Turkey against to stem and bulb nematodes (*D. dipsaci*). Production of cool season food legumes can be severely limited by nematode attack. Symptoms are yellowing, wilting, stunting, decreased biomass and seed yield. The stem and bulb nematode, *D. dipsaci* is an economically important pest that is described as one of the major limiting factors in chickpea production. The main factors for effective integrated control are correct diagnosis of the nematode problems and use of tolerant and resistant cultivars.

Nematodes are a severe limitation of many crops all over the world. Attacked plants have poor root systems and are much more susceptible to biotic and abiotic stresses, such as drought and infertile soil. They may indicate symptoms of wilting, yellowing, stunting, poor seed yield and decreased biomass. Also, nematodes may mix with other soil-borne pathogens increasing the disease severity. They may reduce root nodulation of legumes by rhizobia, limiting nitrogen fixation in cropping systems, and they may reduce the nutritional quality of gram. The cool-season food legumes such as chickpea (*Cicer arietinum* L.) is a host for many species of nematodes. The most damaging species are the root lesion (*Pratylenchus* spp.), stem (*D. dipsaci*), root-knot (*Meloidogyne* spp.) and cyst nematodes (*Heterodera* spp.). On the basis of responses by 371 nematologists in a worldwide survey research (Sasser and Freckman., 1987) has shown that the average yield loss from nematodes is 12.3% for all crops and 13.7% for chickpea,

valued at US\$328 million (Sharma et al., 1992). Controlling nematodes with nematicides is largely uneconomic for field crops, due to this it is necessary to use resistant and tolerant crop species or cultivars of the same species in various farming systems to achieve integrated control.

The impact of nematodes on cool season food legumes has increased, and a number have been undertaken at international and national research centres. This has resulted in greater insights on nematode problems of food legumes and their management, which have been partly discussed in previous conferences (Greco and Di Vito, 1993; Sharma et al., 1994), book chapters (Sikora and Greco, 1990; Riggs and Niblack, 1993) and a monograph (Ali, 1995). The present knowledge of nematode problems of cool season food legumes and integrated control strategies will be discussed here.

2.10.3. Chickpea

The literature (Nene et al., 1996) has shown the numbers of pathogens, particularly the plant parasitic nematodes, associated with chickpea, have markedly increased over the past 17 years. Also, Sharma (1985) indicated that 46 nematode species or genera associated with chickpea all over the world and Ali (1995) listed 97 species mainly from the Indian sub-continent and the Mediterranean basin. Although, some of the reported nematode species were found in the chickpea rhizosphere and there is no certainty that they feed on chickpea. Therefore, emphasis will be placed only on species with confirmed pathogenicity to chickpea. Chickpea (*Cicer arietinum* L.) is an ancient crop that has been grown in India, the Middle East and parts of Africa for many years. Research (Barker & Noe., 1987; Jatala & Bridge., 1990) has shown that *D. dipsaci* is one of the greatest threats to chickpea production after root lesion, root-knot, and *Heterodera ciceri* nematodes.

2.10.4. Accessions of wild *Cicer* species (*C. reticulatum* and *C. echinospermum*) to nematodes for resistance

Brown (1987) showed that by making the initial population density of nematodes small, it can decrease the damage caused by plant-parasitic nematodes and was the best obtained in low-input cropping systems, by growing resistant crops or cultivars. The capacity of the plant to prevent or decrease nematode multiplication was defined as resistance (Trudgill et al., 1988). The body of literature (Cook and Evans, 1987) has shown that resistant cultivars were attractive within cropping rotations, as the direct changing of a susceptible cultivar often requires no specialized equipment and shorter rotations can be applied. In addition, by using resistant cultivars eliminated the need for nematicides. Cook and Evans (1987) indicated that resistant cultivars can be completely resistant (allowing no nematode multiplication) or partially resistant (allowing intermediate levels of nematode multiplication) to related pest or pathogen.

Work (termed Pf; e.g., Fernandez et al., 1994) has shown that resistance is usually measured by detecting the final nematode densities or multiplication rates (termed Pf/Pi) to determine the final population density/Initial population density in plants. The accession of wild *cicer* sp has also been defined as having a multiplication rate (MR), a resistance genotype as having $MR < 1$, and a susceptible genotype $MR > 1$. Evaluation of MR enables a comparison when different initial densities occur and allow comparisons across experiments even between nematode species. Multiplication rates of nematodes can be affected by many factors such as soil moisture, temperature texture, and available nutrients.

Due to this, the multiplication rates were generally estimated in laboratory conditions where temperature, moisture, and soil nutrients were controlled. Moreover, Nombela and Romera (1999) indicated that the acceptance of greenhouse tests with field experiments was essential to better understand how nematode densities may change under different environmental conditions.

2.10.5. Field Experiment

The number of samples and sampling is important for determining the absence or presence of plant parasitic nematode within plots to allow for a correlation with evaluation or resistance in crops host (Ferris Ferris, 1984; Franc 1986 and et al., 1990). Also, physical characteristics such as water or soil type, soil type of field experiment and soil pH can be affected by sampling methodology and nematode distribution. For example, a greater population density of *P. neglectus* was found only in low pH < 6.5 while *Radopholus* was found in regions of pH > 7 in Western Australia. Moreover, when sampling for nematodes, distribution is not hemogenic for most species of nematodes and limits the precision of data in the field experiment.

2.10.5.1. Plant-parasitic nematodes associated with chickpea in Turkey

Recently the population of Turkey significantly quadrupled, increased from 28.1 million people to 80.7 million people during the 1960 to 2017 period. In accordance with this expansion, there is a considerable increase in food demand which has to be supplied by enhancing productivity in croplands or by establishing new production areas. Moreover, chickpeas contain 269 calories, 45g of carbohydrates, 13g of dietary fiber, 4g of fat and 15g of protein which plays a very important role in nutritional resources. Various factors are responsible for a decrease in agricultural productivity in Turkey. Viruses, viroid, bacteria, fungi, weeds, insects and nematodes constitute pests that can cause more than 10% crop loss yearly. This reduction amount is considered to be enough to feed more than 1/10 of the world population, especially in low-income countries. Many soil-borne pathogens and pests cause damage and yield losses in several crops and nematodes are considered as one of the most economically important factors of reduction. The chickpea was established previously in the tribe Viciae of the legume family including Lentil vetch and faba beans. During of vascular anatomy and pollen morphology, the tribe *Cicereae* was separated from another member family of the

Vicieae (Kukicha, 1977). Previously, botanists were beginning to follow the geographical area of chickpea, for example, Vavilov (1926) found two primary areas in the southwest Mediterranean and in Asia. Also, similar studies in the southeastern part of Turkey and neighbouring Syria found the possible source of origin of chickpea-based on the possibility of the closely related annual species, *C. echinospermum* and *C. reticulatum* (Van der Maesen 1987).

Knowledge of the hosts and geographical distribution of important plant parasitic species is essential and important. Most of plant-parasitic nematodes are found in rhizosphere soil and are endoparasitic, but many ectoparasitic species occur in the soil around roots. Some nematodes species are not having a major economic effect when they do not cause significant damage to host crops, however when they do cause significant damage, they are considered a plant pathogen. It is also important to know when population densities of plant-parasitic nematodes inflate to reach is called the economic threshold.

Moreover, it is important to know about the host of a species and the pathology of plant growth and yield. Surveying large regions for the absence or presence of plant parasitic nematodes is not easy but important. Surveying a large area must be selected at random and sampling procedure must be based on standardized sampling methods. The population density of nematodes must be calculated to determine both the quantitative and qualitative event of important species and interaction of their population levels to crop damage. Non-agricultural areas including cultivated land and non-crop plants in and outside the area must also be surveyed to detect potential pest problems. This application will help an understanding of the alternative hosts and appearance of the biology of nematode species including damage symptoms, life cycles, population trends and agroclimatic information on soil types, climatic conditions, cropping sequences. Also, the times of sampling are also important in surveys because populations are dependent on them.

Chickpea (*Cicer arietinum* L.) is an ancient crop that has been grown in India, the Middle East and parts of Africa for many years. Also, it is an important production crop, ranking third amongst the pulse crops (FAO, 2008) and is a good source of many dietary nutrients (Wood and Grusak, 2007). The major chickpea growing countries are India, Pakistan, Turkey, Iran, Myanmar and Iraq in Asia. Chickpea grows in many areas, as a food crop, as a cash crop, and for soil fertility restoration. Chickpea probably originated in southeastern Turkey and spread west and south via the Silk Route (Figure 2. 1). Four centres of diversity have been identified in the Mediterranean, central Asia, the near east and India as well as a secondary centre of origin in Ethiopia. Nematodes cause damage to food legumes in many countries (Greco, 1985; Greco & Vitro, 1988; Greco & Sharma, 1990; Sikora & Greco, 1990).

Figure 2.1. Chickpea growing regions of Turkey (marked in blue).



Information on nematodes infecting chickpea (*Cicer arietinum* L.) was limited. Therefore, a survey was conducted during 2014 to 2016 to identify the nematode association with this crop in Turkey to serve as a basis for future investigation aiming at illuminating the role that these pathogens undertake in the Chickpea production in Turkey.

The root lesion nematodes, *Pratylenchus* spp. and *Pratylenchoides* spp., are most likely the most widespread nematodes in such legume crops as Chickpea (*Cicer arietinum* L.), faba bean (*Vicia faba* L.), lentil (*Lens culinaris medic.*) and alfalfa (*Medicago sativa* L.), in the Mediterranean basin (Grice et al., 1984), root lesion nematode was the most common of the several plant-parasitic nematodes associated with Chickpea. *P. thornei* and *P. penetrans* occurred along the country and were suggested as the cause of reduced crop yields. Other nematodes reported in chickpea were species of *Helicotylenchus*, *Paratylenchus*, *Tylenchus*, *Longidorus*, *Trophurus*, *Trichodurus*, *Xiphinema* index, *X. pachtaicum* (Di Vito M; Gerco N; Oreste G & Saxena M.C, 1998).

The identification of new or potentially harmful species of nematode in the Chickpea is important to the success of agriculture and aids in the development and evaluation of quarantine or regulatory procedure to minimize their spread. The correct species identification is a prerequisite to efficient nematode control and successful plant quarantine operation. Also, similar studies in southern Spain observed that a survey in chickpea crops (*C. arietinum*) showed that the legume and cereal root lesion nematodes such as *P. thornei* and *P. neglectus* were the most important and widespread plant-parasitic nematodes. These nematodes were collected and recovered from soil (90%) and root samples (84%) with a population density of 2-48 nematodes from 500g of soil and root.

2.10.5.2. Extraction of nematodes

Extraction methods to identify the population density of plant parasitic nematodes are essential for the soil of plants. Also, for detecting densities of nematodes accurate quantification from soil and plants is necessary. Due to this, Feriis (1987) defined two terms for the extraction methods of nematodes from soil and plants, being catch efficiency and separation efficiency. Catch efficiency is the extraction of nematodes by using the tools and methodology that is available and separation efficiency is the proportion of the nematode population by using of the

special technique, tools and by measuring the percentage of recovery from repeated extraction from the same sample to separate nematodes from plant or soil. Common methods used for recovery of plant-parasitic nematodes from plants include the misting method (Seinhost, 1950) and the Baermann method (Baermann, 1917) which are used for extraction of nematodes from plants.

Extraction methods of nematodes from plants can also be obtained using maceration (Dunn 1973; McClure et al., 1973) or centrifugal sugar flotation (Greco and D Addabo, 1990). These extraction techniques are very useful for extraction of mobility nematodes such as migratory plant-parasitic nematodes however is not suitable for the extraction of eggs and sedentary species.

2.10.5.2.1. Research importance

Variation in the virulence of populations of RLN, *P. neglectus*, *P. thornei* and *D. dipsaci* nematodes were important when considering the estimation of yield loss, and especially for the improvement of tolerance or resistance varieties, as well as measurable differences which may help to detect the benefits and longevity of each variety produced. Before this current study commenced, there was no further information about the biology of migratory endoparasitic nematodes such as *P. thornei*, *P. neglectus* and *D. dipsaci* and the population density of these nematodes in the growing soil and growing areas of chickpea in Turkey.

To identify the effect of root lesion nematodes (*P. thornei*, *P. neglectus*) and *D. dipsaci* in this region, information was required on the resistance and tolerance of several crops and cultivars, sampling methods and strategies in soil and plants, levels of yield loss caused by this species of nematodes and variability within and between Turkey population of *P. thornei*, *P. neglectus* and *D. dipsaci*. To establish the yield loss caused by plant-parasitic nematodes, techniques for both the management of population density of nematodes and exact determination nematode density must be assessed. This study evaluated the use of wild *Cicer* spp. accessions (*C. arietinum*, *C. reticulatum* and *C. echinospermum*) against to both

root lesion (*P. thornei*, *P. neglectus*) and *D. dipsaci* for determining yield loss caused by these nematodes to find resistance cultivars in Turkey (Table 3). The sampling and extraction methods for estimating the density of these nematodes from soil or root were assessed.

2.10.6. Population dynamics of the root lesion nematodes *P. thornei* (Sher and Allen), *P. neglectus* and *D. dipsaci* on different species of chickpea.

The study population dynamics of the root lesion nematodes *P. thornei* (Sher and Allen), *P. neglectus* and *D. dipsaci* is very important. The main aim of this study is to determine and identify population dynamics of root lesions (*P. thornei* and *P. neglectus*) and *D. dipsaci* in chickpea fields. The field trials to assess the effect of varieties on population dynamics of the RLN (*P. thornei*, *P. neglectus*) and *D. dipsaci* is important due to its patchy distribution in the soil. Every change in the population density of nematodes is commonly estimated by assessing either final nematode density (Pf) (at or near plant harvest time) or the multiplication rate (MR) that is calculated by dividing the final nematode density by initial density (Pf/Pi).

Elekcioglu and Gozel (1997) reported that the numbers of *P. thornei* in the soil were low until March and then increased during the growing season in Adana Province, Turkey. Imren et al., (2017) and Sahin et al., (2008) have shown a correlation between soil temperature and *P. thornei* populations in that nematode populations were low from November to March/April and increased to June/July in Ankara Province, Turkey. The initial sampling was in March 2015 - 2017 during the cold season through to the end of growing season in July in the Ankara province.

3. MATERIALS AND METHODS

1. Methodology

- a. Methodological studies to adapt the chickpea assay to suit *Cicer*. This optimization includes tested soil types, inoculation volume, nematode density and incubation time (eg. 12, 16 or 24 weeks) in *C. reticulatum*, *C. echinospermum* and *C. arietinum*.
- b. Isolation of *P. thornei* and *P. neglectus* from chickpea fields in Southern and Eastern Anatolia (Urfa, Diyarbakir, Gaziantep). Sample soil and plant roots.

2. Screening

The researchers aimed to screen the whole collection as the 1,177 wild *Cicer* accessions that were collected since 2013 within the nematode screening capacity of Cukurova University over a 3 year. Accordingly, the researchers began supplying germplasm from Harran to Cukurova University as soon as the multiplication was complete.

Table 3. The list of wild *Cicer* genetic resources since 2013, 2014, 2015 (accession and site numbers).

Year	<i>C. arietinum</i>		<i>C. bijugum</i>		<i>C. echinospermum</i>		<i>C. pinnatifidum</i>		<i>C. reticulatum</i>	
	Sites	Acc.	Sites	Acc.	Sites	Acc.	Sites	Acc.	Sites	Acc.
2013	-	-	4	68	6	96	1	4	15	294
2014	1	1	1	8	2	76	-	-	20	221
2015	-	-	-	-	8	100	23	239	6	72
Total	1	1	5	76	16	272	24	243	41	587

- a. Quantify nematode dynamics over growing seasons using species subsets from the Ankara and Urfa GxExTOS trials. Nematode numbers were quantified monthly over the entire growing season in samples taken from

plant roots and nearby soil. These destructive harvests were accommodated in adjacent small trials that will also be used to calibrate plant growth measures (min and max diameter) against canopy development estimated by photograph, leaf area, and dry matter measurements.

In addition, these projects aim to widen the genetic and adaptive diversity of chickpea by collection, characterization, evaluation, and exploitation of wider *Cicer* genetic resources. The researchers' contribution to this study is to find RLN resistance by screening more than 400 genotypes against *P. neglechtus*, *P. thornei* and *D. dipsaci* for resistance. Furthermore, characterization of the 2013-2015 collection site soils in order to establish the nematode taxonomy and population density at the point of the collection was assessed. By combining this data with a recent survey of domesticated chickpea soils performed, it becomes feasible to generate a nematode survey across the domestic and wild *Cicer* distribution range in Turkey. Interestingly, *Pratylenchus* spp. were relatively rare compared to foliar nematodes such as the *Aphelenchoides*. When the data assembly is complete the researchers will collaborate on multivariate analysis and mapping. Also, use of soil samples to generate a *Cicer* nematode test population to ensure that the *Cicer* germplasm is challenged against the appropriate nematode were assessed. This entails isolating *P. thornei*, *P. neglectus* and *D. dipsaci* from chickpea crops and culturing these on carrot disks using sterile techniques.

This study was conducted to give to helpful management systems for reducing the effect of both RLN (*P. thornei*, *P. neglectus*) and stem and bulb nematode (*D. dipsaci*) in broadacre cropping systems in Turkey.

3.1. Laboratory Experiment

3.1.1. Extraction of nematodes from plant material

Plant-parasitic nematodes are present in almost any type of plant material. Nematodes are not just found in soil and root also in bulbs, stems, leaves, tubers, and seeds. Also, materials such as moss, litter, and compost often contain a high number of saprophagous nematodes (non-parasitic). Extraction methods are generally based on nematode mobility in plant material.

Because of their mobility, it is possible for living nematodes to be separated from other particles. When a sample is placed on the sieve with a moist filter paper positioned in a shallow water-filled tray, migratory endoparasite nematodes such as root lesion and stem and bulb nematodes move from the sample into the water where nematodes are collected in a clear suspension.

Many extraction techniques of nematodes from plant or soil were based on a combination of these principles and established based on the modified Baermann Funnel method (Baermann, 1917). The advantage of this method is simple and cheap when extracting plant-parasitic nematodes from plant materials, the final suspension is virtually clean. The performance of extraction is rather high for small samples. Among nematology laboratories, many extraction methods have been used but the Baermann method is one of the most effective extraction methods compared to other extraction techniques (Prot et al., 1993).

3.1.1.1. Structure of misting chamber-The double funnel method

In this method, plant or soil materials were placed in a funnel that holds a sieve and a filter paper. The funnel end was covered by rubber tubing with the clamp at the bottom. Also, water was added to cover the soil or plant tissue. Migratory nematodes such as RLN and stem and bulb nematodes move through the filter paper to the bottom. After 24-48 hours, endoparasitic nematodes were collected by emptying 5-10 ml of water by removing the clamp from the tubing in

a shallow dish (Agrios, 2005). After extraction, the soil and plant water suspension were separated from the nematodes.

Figure 3.1. The Dubble funnel method for extraction RLN (*P. thorne*, *P. neglectus*) and *D. dipsaci* from root materials (Bohmer and Weil, 1987).



The misting chamber and equipment: To keep the soil and plant samples, baskets with 9 cm in diameter and 7 cm in height were established by PVC plastic covering one end with plastic fly wire (Figure 3.2). The baskets kept on the inner funnel end of each basket lining. The inner funnel was rested on the outer funnel and outer funnel (10 cm diameter) was modified by fixing a hard plastic to the base of the funnel to which flexible tubing (11cm length by 6 mm outer diameter) was attached.

The misting chamber was set up by brass spray cone jets 15 cm above the funnels. Also, above every four funnels located one jet, with the centre of each funnel 12 cm apart. Between spray from each jet, there was minimal overlap. The temperature of the water was arranged to be 22°C at the surface of the sample. To use a misting protocol helped to maximize the number of nematodes recovered from each sample, produce the cleanest samples and reduced the number of nematodes in the over flow.

Figure 3.2. Misting chamber for the extraction of *P. thornei*, *P. neglectus* and *D. dipsaci*.



3.1.1.2. The Baermann petri method

Originally, the researchers used this method for extraction and recovery nematodes from soil and plants. This method is based on the Baermann funnel method and is based on the same principle systems that nematodes are moving from the samples to the water. Petri dishes (the one with a sieve at the bottom) were used instead of funnel. In this way plants or soil materials were placed in a dish and after water was added. After 24-48 hours, the water of dishes containing nematodes were collected (Eppo., 2013).

Figure 3.3. The Baermann method used the extraction of *P. thornei*, *P. neglectus* and *D. dipsaci* growing in the soil of chickpea fields and the plants themselves.



Figure 3.4. Petri dishes including 100gm soil sample use for extraction of nematodes in the Baermann method.



3.1.1.3. Extraction of nematodes from soil and plant roots

For extraction of nematodes from plant roots, the first roots were washed completely under running tap water and then cut into 1 cm in lengths. In the final, a maximum weight of 5 g fresh weight of root was placed in Petri dishes. After 24-

48 hours, samples were misted (with tap water) for 15 second every 10 minutes, then a small volume (about 10-15 ml) of water containing nematode was released from the tubing and stored in 50 ml special of falcon tube at 4°C prior to counting. Nematodes were extracted from 100 g of soil samples using the Baermann method, as shown in Figure 3.4, 3.5 and 3.6. Before placing soil samples in petri dishes, large soil clumps were broken by hand and soil was equally distributed over the tissue. Then, tap water was added to the soil sample. Samples were ready to extract after 24-48 hours. The nematode suspension was concentrated by passing water and the nematodes were washed off the sieve in 10 ml water and stored at 4 °C in a falcon tube before counting.

Figure 3.5. The Baermann petri dishes method for extraction nematodes.



3.1.1.4. Counting and identification of nematodes

The samples containing nematodes were counted in modified Doncaster (1962) dishes using a light microscope at either 40x or 20x magnification. When the nematode density was higher (> 200 nematodes/samples), samples were scaled

to identify volume, then 1 ml of subsamples were taken and the population density of nematodes per ml was counted (Figure 3.7).

Also, for lower population density of nematode densities, the entire sample was counted. RLN spp. were detected and identified based on the position of the vulva in adult females (*P. thornei* V= 73-80%; *P. neglectus* V=81-86%) (Loof., 1991).

Figure 3.6. Counting and identification of nematodes under light microscope.



3.1.2. Preparation of nematodes for inoculation

3.1.2.1 Carrot culture inoculum

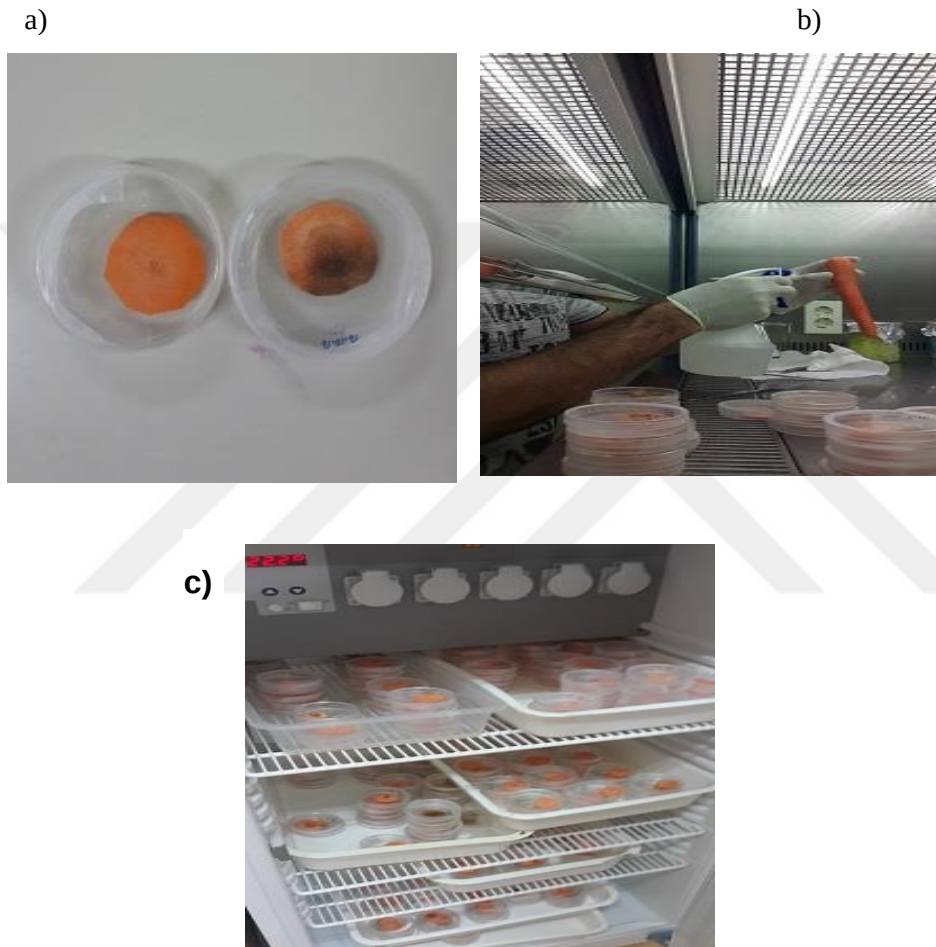
The survey of chickpea crops in Turkey showed that the cereal and legume root lesion (*P. thornei* and *P. neglectus*) and *D. dipsaci* were the richest and most widespread plant parasitic nematode in the region. For the carrot culture, nematodes were collected from 90% of soil samples and 84% of root samples, with the population density of 2-70 nematodes/100gm of soil and 10-50 nematodes/g of the root. A large number of root lesion nematodes eggs, juveniles, and female are

required for pathogenicity of nematode researchers on chickpea. The use of carrot culture disks is one of the best ways for the reproduction of the root lesion nematodes (Huettel., 1985). Different studies have been published about the culture of root lesion nematodes on carrot disks (Chitambar and Raski., 1985; Kaplan and Davis., 1990; Verdejo and Pinoche., 1991). But there was no further reported studies about the culture of *D. dipsaci* on carrot disks and no reference reported about factors such as the effect of time, temperature, and inoculum density on nematode reproduction which is examined and reported below.

P. thornei or *P. neglectus* were produced on carrot cultures by using the method described by Nicol and Vanstone (1993). Every carrot culture disk was established with up to 40-50 individual female extracted from soil or plants. To establish the carrot culture disks, nematodes were surface sterilized in a solution of 4% streptomycin/4% penicillin for 2 hours then rinsed 3 times in sterile water. Nematodes were then added to the callus using a flamed needle to transfer an individual or female nematode to a 50 microliter drop of sterile water on the callus.

After inoculation of nematodes on the callus disks, cultures were stored in dark incubators at 19 -22 °C and observed weekly for the presence of nematodes. Production of nematodes per culture can be affected by factors such as time, temperature and initial species of nematode. Most commonly, carrot culture takes about 2 to 3 months for endoparasitic nematodes such as *P. thornei*, *P. neglectus* and *D. dipsaci*) to produce up to 5000 nematodes per culture. After 2 or 3 months, nematodes were extracted from cultures by dividing them into small pieces then misted. The nematode suspension was collected via the Baermann Petri method and after 24 hours the nematode inoculum was complete by subsequent dilution of the resulting suspension.

Figure 3.7. Carrot culture for the production of root lesion nematodes and *D. dipsaci* inoculum) culture on the right shows a large number of nematodes clumping on carrot disks and the left shows carrot with no nematode production on a carrot. b) Preparation of nematodes with carrot culture technique in the sterile cabinet. C) Stored culture in dark incubator for about 2 or 3 months at 19-22°C.



3.1.3. Growth chamber experiments

Selecting a good chamber of growth experiment is essential to getting quality results. Homogenic factors such as temperature, planting tube size, light, and planting soil are very important in laboratory experiments.

3.1.3.1. Planting and growing conditions

- Fill small pots (plastic pots, the diameter of 30 cm x 30 cm deep) with an autoclaved mixture of 3:1 soil and sand (Figure 3.12)
- Scarify the seeds by making a small cut in the seed coat to improve water absorption and germination (Figure 3.9).
- Dress the seeds with fungicides and insecticides (Figure 3.10)
- Sow at least 50 seeds per accession with two seeds per pot at a depth of 3–5 cm (Figure 3.13)
- Water the pots immediately after sowing and then once every 3 days depending on evaporation rate.
- Starting from flowering, validate each accession against RLN (*P. thornei* and *P. neglectus*) and *D. dipsaci* (Figure 3.13).

3.1.4. Germination of seed

It is necessary to scarify the seeds by making a small cut in the seed coat before germinating to improve water absorption and germination in the wild *Cicer* spp. (Figure 3.9). The individual chickpea seeds were disinfected by hypochlorite (4%) and alcohol (30%) (Plate for pre-germination and placed them on the surface of wet filter paper at 21°C for 3 days in sterile Petri dishes (Figure 3.9 and 3.10).

Then, germinated seeds were planted in the open-ended standard small tube (16 cm in high 2.5 cm in diameter) that contained the field soil, (73% clay, 16.5% silt and 10% river sand) (Figure 3.12 and 3.13).

Before sowing the plants, the field soil was sterilized by an autoclave machine for 2 hours at 121°C. One week after planting, the nematodes were transferred to room temperature (20-25°C) environment and each plant was infected with 1 ml of water consisting of nematodes. In the week after the nematodes were inoculated, all of the plants were simultaneously grown in a

growth chamber at 24°C, 50% humidity under 16/8 h day/night by high-pressure sodium lamp to increase the capacity of nematode influence. For each experiment, selected germinated seeds of the same size and root length (Figure 3.11 and 3.13). A single seed was grown in tubes and supported by a box frame (Figure 3.12).

Figure 3.8. Scarifying wild chickpea seeds by making a small cut in the seed coat to improve water absorption and germination.

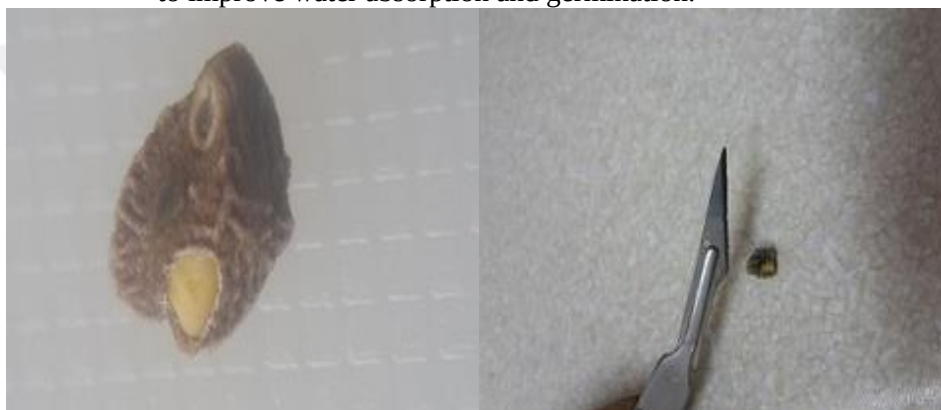


Figure 3.9. Sterilization of seed inside sterile cabinet.



Figure 3.10. Preparation of seeds for planting.

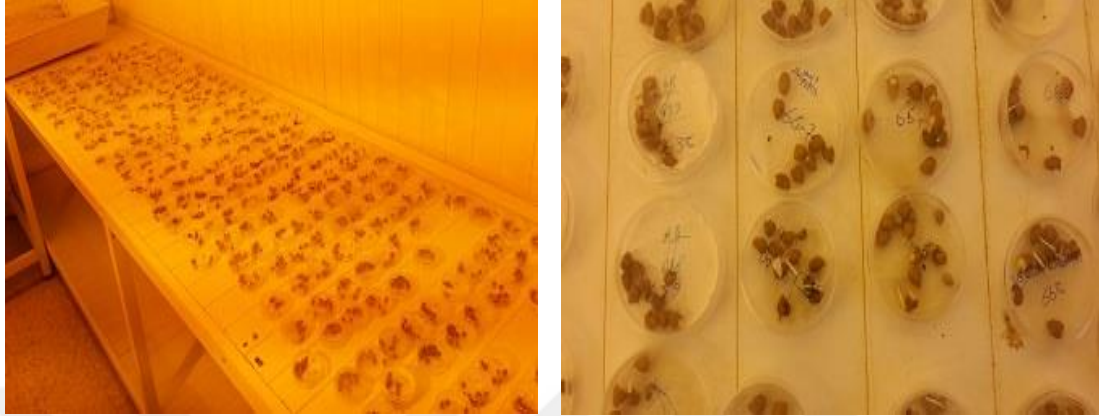


Figure 3.11. Crates (and seedling tubes) used in the laboratory experiments.



Figure 3.12. Controlled environment growth room for the evaluation of pathogenicity of RLN (*P. thornei* and *P. neglectus*) and *D. dipsaci* in chickpea.



3.1.5. Optimisation

Factoring experiments to detect the best integration for screening Chickpea varieties with nine accessions of wild chickpeas (*C. reticulatum* and *C. echinospermum*) were selected for resistance against *P. thornei*, *P. neglectus* and *D. dipsaci* and replicated four times under glasshouse conditions (Table 4).

Table 4. Factors used to control the best conditions for screening chickpea varieties for resistance to *P. thornei*, *P. neglectus* and *D. dipsaci*.

Factor	Control
Inoculum density and Nematode extraction	150, 225 and 300 nematodes/plant for <i>Pratylenchus thornei</i> and <i>P. neglectus</i> . 150, 300 and 400 nematodes/plant for <i>Ditylenchus dipsaci</i> species.
Rating time	16 and 20 weeks
Experiment tube size	Large (40×100 mm), medium (30×100 mm) and small (16×100 mm)
Planting media	Loamy soil (29:70:1- sand (S), field soil (F) organic matter (O) and sandy soil (70:29:1-S: F:O)

3.1.5.1. Nematode inoculum

To in vitro rear *Pratylenchus* spp, carrot discs were used to grow the nematodes. This technique is workable for migratory nematodes (*Pratylenchus* spp.) and *D. dipsaci* for the serial manufacture of nematode for experimental aim, direct study of nematodes, screening trials and to increase nematodes. The greatest advantage of this method is to save time with a quick mass production of nematodes. The literature (O'Bannan, 1968) has shown that this method helped the *Pratylenchus* spp nematode to be produced on sterilized carrot discs in Petri dishes in an incubator more than in a root plant under laboratory conditions. For a more complete collection, the carrot disc was first cut into very small pieces and placed in the Petri dishes with sterile water which let the nematodes migrate freely into the Petri. This enabled the extraction of nematodes to be counted under a binocular microscope and then placed in the flask.

3.1.5.2. Chamber of growth

Single chickpea seeds were disinfected by hypochlorite (4%) and alcohol (30%) for pre-germination and placed on surface wet filter paper at 21°C for 3 days in sterile Petri dishes. Then, the germinated seeds were planted in a standard small tube (16 cm in high, 2.5 cm in diameter) that contained the field soil (73% clay, 16.5% silt and 10% river sand). The researchers used inoculum densities of 150, 225 and 300 for RLN and 150, 300 and 400 for *D. dipsaci* nematode. Before sowing the plants, the field soil was sterilized by an autoclave for 2 hours at 121°C. One week after planting, the nematodes were transferred to a room temperature (20-25°C) environment where each plant was infected with 1 ml water consisting of 150, 225, 300 nematodes separately in 1 ml water for lesion nematodes and 150, 300 and 400 nematodes for *D. dipsaci*. In the week of nematode inoculation, all of the plants were simultaneously grown in a growth chamber at 24°C, 50% humidity under 16/8 h day/night by high-pressure sodium lamp to increase the capacity of nematode influence.

3.1.5.3. Nematode extraction

The trial was conducted in a randomized complete block design with 4 replicates and all of the treatments tested at the same time. After sixteen and twenty weeks since the nematode injection, plant shoots were removed and the nematodes were extracted from both of roots and soil using the Baermann technique. Therefore 1 ml of nematode suspension was counted with four replications in a counting slide under a binocular microscope then the number of extracted nematodes per plant was calculated.

3.1.6. The carrot culture of *Ditylenchus dipsaci*

For the carrot culture, nematodes were collected from 90% of soil samples and 84% of root samples, with the population density of 2-70 nematodes/100gm of soil and 10-50 nematodes/gm of root. A large number of *D. dipsaci* nematodes'

eggs, juveniles, and females are required for pathogenicity of nematode research on chickpea. The researchers chose the infected roots and the growing soil of the chickpea with *D. dipsaci* for extraction of the nematode to be the culture. Nematodes were inoculated on the carrot disc.

3.1.6.1. Experiment 1

The effect of time of incubation period on the population density of *D. dipsaci* nematode and reproduction was assessed at 20, 40 and 90 days. After inoculation, by adding 50 surface-disinfected female and males to each disk species of *D. dipsaci*, nematodes were transferred by a sterile handling needle into a drop of sterile distilled water that was placed on the carrot disk. These disks were incubated at $19^{\circ}\text{C} \pm 1^{\circ}\text{C}$ in the dark. For determination of each incubation period on the nematode, Inoculum disks were placed in the incubator in a completely randomized design with 10 replicates.

3.1.6.2. Experiment 2

The effect of temperature on nematode reproduction was assessed at 15°C , 20°C , 25°C and $30^{\circ}\text{C} \pm 1^{\circ}\text{C}$ with an inoculum density of 50 female and males (1:1) *D. dipsaci* nematodes per disk. For these purposes, nematodes were transferred to carrot disks for study. Inoculated carrot disks were incubated at suitable temperatures in the dark for 35 days. In addition, for each temperature there were 10 replicated disks and disks were completely randomized within each incubator.

3.1.6.3. Experiment 3

The effect of initial inoculum density on nematode reproduction was studied using 50, 100, 200 and 300 surface-disinfected nematodes (all life stage) per disk. The inoculum density of nematodes was estimated in a 1 ml subsample of the extracted nematodes and was transferred to carrot disks as a suspension form in sterile water. The medium average of life stage in the inoculum density of

nematode was 16.2%, 56.9% juveniles, and 26.9% female. All of the inoculated carrot disks were then incubated at $20^{\circ}\text{C} \pm 1^{\circ}\text{C}$ in the dark for 35 days. Each inoculum density disks were placed in the incubator in a 10 replicate with a completely randomized design.

The suggested temperature for rapid multiplication was between 19°C and 23°C , depending on nematode reproduction population and must be sub-cultured every 4-6 weeks. The development rate decreased when temperature was increased up to 30°C or decreased down to 18°C . To first start a carrot culture, clean carrots were selected without being overly thick and of having visible cracks. The selected carrots were washed under distilled water, and all equipment and materials sterilised by autoclaving at 121°C for 20 minutes and sterilised the working and tools surface with 96% ethanol. The carrot was held with 96% ethanol and flamed over a spirit lamp. This was repeated three times.

Also, all of the equipment was sterilized including forceps, peelers, and knives with ethanol and were each flamed before use. The carrot was peeled with the sterilized peeler and was cut into 5 mm thick sections of 3-4 cm diameter and transferred into sterilized Petri dishes by using the sterilized forceps. The carrot disk must not be less than 3 cm in diameter, then the carrot discs were transferred into sterilizing glass Petri dishes (5-6 cm diameter). They were stored in the dark incubator to keep in underground condition for 3-4 weeks at $19-23^{\circ}\text{C}$. Also, it was necessary to carefully check the carrots every week to observe the best signal of healthy cultures during incubation. After 3-4 weeks the nematodes started to exit the carrot and when nutrient in the carrots became empty it was an indicator to harvest the nematodes.

In addition, the incubation time and the initial inoculum effected how soon the nutrients become empty. For a selection of nematodes, a concentration of the nematode extract was placed onto a small (3-5 cm diameter) petri dish and accounted for under a light microscope. The number of nematodes required for inoculation depends on how many carrots are to be utilised. However, it was

suggested to inoculate at least 100-150 nematodes on one carrot disc and not inoculate less than 100 of them. It was recommended to inoculate at least five replicates from Petri dishes. For mass multiplication, the number of inoculated nematodes increased on one carrot disc and selected female and male of the *D. dipsaci* nematode were placed in a glass Petri with sterile distilled water. The aim was to deliver 50-80 nematodes per disc in a maximum of two 50 microliters of nematode suspension to transfer the nematodes.

The number of nematodes in the suspension was determined the number to transfer. It was suggested use about 80-150 nematodes for mass multiplication. After that, carrot disk was placed in an incubator for a 5-6 weeks, then the nematodes were harvested and stored in the fridge at 5°C. The nematodes can remain viable for up to 1-2 weeks.

For a more complete collection, carrot discs were first cut into tiny pieces and placed in the Petri dishes with sterile water which let the nematodes migrate freely into the Petri, then, the extracted nematodes could be counted under a binocular microscope and placed in the flask.

3.1.7. Experimental design and analysis

A laboratory experiment was set up in a randomized complete block design. The data was analysed according to standard analysis of variance procedure with Gen Stat (Statistical and Mathematical software) discovery edition with statistical differences considered significant at $P \leq 0.001$.

3.2. Field experiment

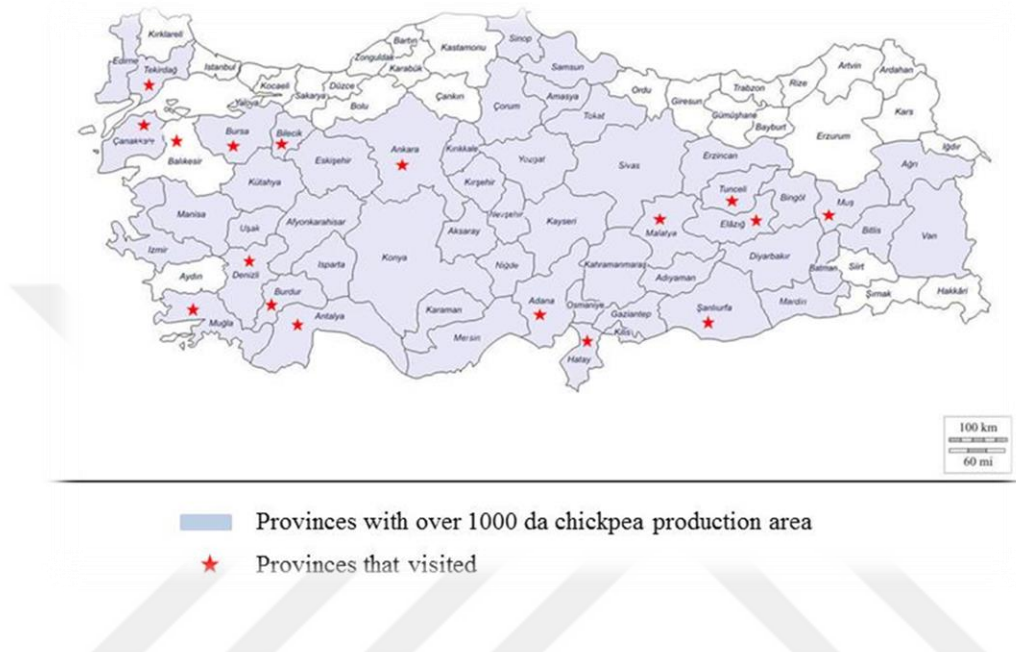
3.2.1. Nematode survey

A nematode survey was carried out over a period of 17 locations in 37 districts in different chickpea growing areas of Turkey in 2014 to 2016. The distribution map of the sampling location is presented (Fig 3.14). A total of 211 soil and root samples (74 in 2014, 69 in 2015 and 68 in 2016) were collected from

37 different regions from the soil of a chickpea field at depth of 0-30 cm, plus, the root of Chickpea plant (Table 4). The soil was sampled by coring tubes (2.5 cm by 10 cm) from the soil to a depth of 0-30 cm. Approximately 500g of soil was taken from a depth of 0-30 cm from chickpea fields.

For the evaluation of nematodes in field plots, 5-10 subsamples were taken using a soil Auger at a depth of 0-30 cm and stored to produce a 500 g composite sample. Some subsamples were taken from each location and then one composite sample was made from them. Then, the soil samples were transported to the laboratory after collection and stored at 4°C prior to cultivation. In the laboratory, plant shoots were removed and nematodes were extracted from 500 g of subsamples by Cobb's sieving, centrifugal flotation (Jenkins, 1964) and Baermann funnel method, and also from the roots by using incubation (Young, 1954) or Coolen's technique (1979) then heat killed at 60°C for one minute, fixed in a TAF solution and mounted on slides by wax-ring method (Seinhorst, 1959). The identification of species was carried out based on morphology and morphometrics of female individuals. The permanent slide was examined by a light microscope for species identification. Handoo and Golden the keys (1989) was used to identify *Pratylenchus* spp.

Figure 3.13. Distribution map of the sampling locations of chickpea growing areas in Turkey.



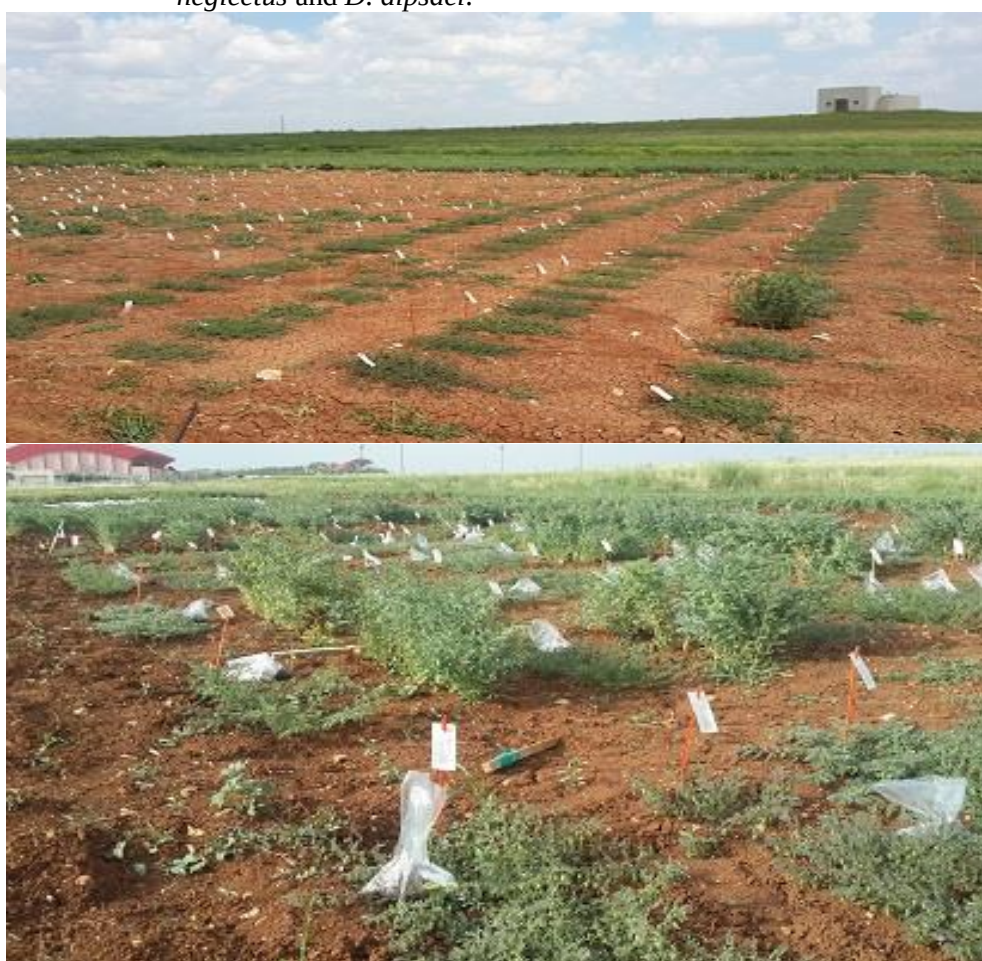
3.2.2. Choice of environment and planting season

Chickpea is a cool season food legume that can be sown and grown in different climates, from the temperate to semi-arid environment. The planting season is different between areas. In a Mediterranean environment such as Turkey, this coincides with the winter season. Experiments were established at the beginning of March during the 2015–16 and 2016–17 chickpea growing season.

The field experiment was conducted in two growing chickpea fields in Ankara and Sanliurfa. The examination field in Urfa was located at Harran University (altitude: 465 m; 37° 08 N and 38° 46 E) where the climate changes from arid to semi-arid and between May to September. In this location, an average rain of 460 mm was recorded for each year and the relative humidity was found to be about 49%.

Also, the weather was usually warm during the winter in the second investigation site, located on the Haymana (Ankara) at the intersection of southern Ankara and in a position that the centre could be considered as 39 degrees north and 32 degrees east where chickpeas are planted between March and April and harvested in June/July (Figure 3.15).

Figure 3.14. The field study for the evaluation of resistance to *P. thornei*, *P. neglectus* and *D. dipsaci*.



3.2.3. Seed preparations for planting

After receiving accessions of wild *Cicer* spp, the seeds were divided from each accession into a subset of 120 seeds for every 4 m long row for a four-row plot. For each subset of seeds, a packet was prepared which had the accession of wild *Cicer* spp. written on it and the number on each packet. Then, the sterilized and treated seeds with appropriate insecticide and fungicide (sodium hypochlorite, KMnO₄ and H₂O₂ with washing by water after each one gave best results for beans) and the prepared seed for planting was placed in each subset of seed. The labelled packets were placed with the gene bank label on top and additional packets underneath stapled together to create four packets of 120 seeds for four rows.

3.2.4. Field selection and preparation

Planting in a well-drained soil and being sure of a good reserve of soil moisture is one of the most important factors in a field experiment. The seeds were planted in a normal soil type with a PH 7.5 for best results. Also, two harrowing were followed to invert the soil to produce a fine and flat seedbed.

3.2.4.1. Planting layout, distance and density

Seeds were planted in four rows with 120 seeds per row, 4 m long, for accessions that are populations of genetically diverse materials, such as landraces. Also, a distance of about 60 cm was left between rows to allow enough space for inter-row cultivation and isolation distance of 90 cm between plots. For the sown seed, a machine designed for purpose was used and the seeds were directly sown into a flat seedbed at depth of 5 cm and cultivated one seed every 4 cm. Also, it was ensured that the planter was free of residual seeds when moving onto the next accession. Moreover, when planting was by hand, the furrows were opened to about 5 cm depth and seeds were placed to be 4 cm apart. The furrows were then closed once completed and plastic labels were used to mark each plot to be marked with plot number and the accession number for identification.

3.2.4.2. Apply for irrigation

In both years, all treatments were irrigated by sprinkler system. Irrigation applied immediately after sowing, preferably by drip system or sprinkler, with application of supplemental irrigation 10 days after planting without rain to ensure a competent seed yield. Also, plants should not become so water-stressed when flowering as pod abortion occurs or the pod-filling is impeded and avoided due to excessive soil moisture during the cropping season. Harvesting time is when the colour of leaves turns yellow and pod becomes dry.

3.2.4.3 Soil sampling

Soil samples were collected at 30-day intervals from March 2015-2016 until July 2016-2017 from eight plots (7.2 m wide x 2 m long =14 m²) per field. Each plot consisted of single rows 2 m long and distances between the rows were 60 cm and inter row spacing was 5 cm. The seeds were sown at a 5-6 cm depth. The population densities of four nematodes were assessed on the winter and spring chickpea during the 2015-2016 and 2016-2017 growing seasons.

Data on the number of each nematode species in the soil samples were collected from subsamples taken during two consecutive seasons and five samples for five consecutive months were taken from each experimental site (Ankara and Urfa) in both experiments. After mixing the samples, 100gm of subsamples were taken and the nematodes were extracted by the modified Baermann funnel method from the mineral soil fraction and the root fraction separately (Hooper 1986).

3.2.4.4. Soil sampler

The soil was sampled by cylinders that were placed tightly on the soil surface and beaten into the soil to a depth of 10 cm and 20 cm. Then, the cylinders were pulled free or by using a commercially available coring tube (2.5 cm by 10 cm) or a soil Auger by 5 cm diameter. For evaluation of the nematodes in field plots, 5-10 subsamples were taken using a soil Auger in depth of 10-20 cm soil and

stored to produce a 500gm composite sample. Samples were taken from each plot within one week following seeding (for determination of initial population density) and within 4 weeks (each month) either pre or post-harvest (final) (Figure 3.16). Finally, soil samples were transported to the laboratory after collection and stored at 4°C prior to cultivation. The soil was sampled by using an earth Auger or undisturbed core (Figure 3.16).

3.2.5. Population density of nematodes

Because the percentage between the numbers of nematodes in the mineral soil fraction and the root fraction changed during the growing seasons, the numbers of root lesion nematodes found in the mineral soil fraction cannot be used to judge the total number of nematodes in the soil. Therefore, both fractions must be processed to make a true estimate of the density. Nematode counts were performed on each soil sample. Populations density of nematodes were commonly estimated by assessing either final nematode density (Pf) (at or near plant harvest time) or multiplication rate (MR), that was calculated by dividing the final nematode density by initial density (Pf/Pi).

Figure 3.15. Equipment used for sampling soil.



Figure 3.16. The sampling of soil by using soil Auger by 5 cm diameter in 10-20 cm soil depth.



3.2.5.1. Population dynamic density of nematodes

In this study, 12 accessions of wild *Cicer* sp (*C. reticulatum* and *C. echinospermum*) of winter and spring chickpea cultivar evaluated the occurrence and population dynamics of the root lesion (*P. thornei* and *P. neglectus*) and *D. dipsaci* nematodes with four replications. The experiment evaluated the population dynamics of density increase of root lesions (*P. thornei* and *P. neglectus*) and *D. dipsaci* in *C. arietinum* (n=3), *C. echinospermum* (n=4) from 3 collection sites; and *C. reticulatum* (n=5) from 3 collection sites. This study was conducted during 2014-2016 at the Field Research Facility of the Faculty of Agriculture at Harran University in Sanliurfa and Haymana region in Ankara, Turkey. The investigational field was located on the Harran Plain (altitude: 465 m; 37°08 N and 38°46 E) where the climate changes from arid to semi-arid and the weather is hot and dry from May to September where temperatures can reach up to 46°C, and is usually warm during the winter. An average of 460 mm of rain falls each year and the relative humidity averages about 49%. The second experimental site was located on the Haymana plain. It is located at the southern intersection of Ankara and in a side position where the centre could be considered as 39 degrees north latitude and 32 degrees east longitude, where chickpea is planted in March /April and harvested in June/July. Experiments were established at the beginning of March during the 2014–2015 and 2015–2016 chickpea growing seasons. At the beginning of experiment, land was plowed and cultivated, then prepared for planting with a single pass of a disk harrow.

3.2.6. Statistical analyses and experimental design

The data (numbers of nematodes per 100gm of dry soil) from the experimental plot were analysed using ANOVA, according to standard analysis of variance procedure with Gen Stat (Statistical and Mathematical Software) discovery edition. Significant differences between treatment and replication of data were calculated at $P < 0.001$.

3.2.6.1. Data analysis and transformation

If residuals plotted against fitted values produced skewed data, (showing large variation within replicates) it is necessary to transformed and normalize the data sets prior to analysis. Where the standard deviation was proportional to the mean Log (x) or (x+1) transformation was used. Also, where data consisted of a number of nematodes recovered from subsamples, the samples square root transformation was considered suitable. The general analysis of variance (ANOVA) was used for statistical significance of difference between treatments that were separated using either Fishers protected least significant difference (L.S.D) from a general analysis of variance (ANOVA) (Gomez and Gomez, 1984) for calculation of the distribution of plant-parasitic nematodes in field plots or the detection of the relationship between final density and initial density of RLN (*P. thornei* and *P. neglectus*) and *D. dipsaci*.

4. RESULTS

4.1. Laboratory experiment

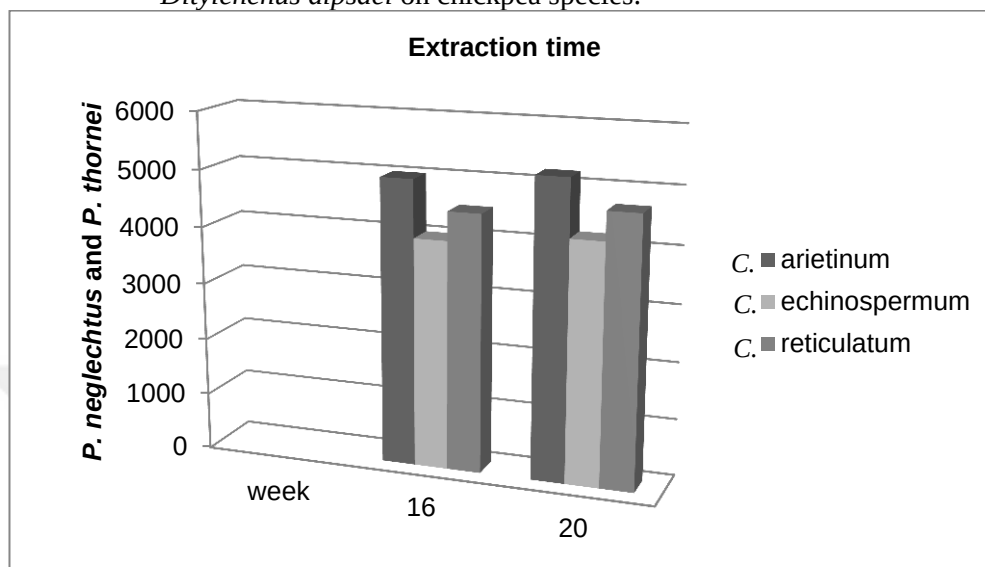
4.1.1 Screening and optimization of chickpea genotypes against the root lesion nematode (*P. neglectus*, *P. thornei*) and *D. dipsaci*.

The aim of this study is to establish a sound methodology for assessing wild chickpea accessions and a screening method for chickpea varieties against *P. thornei*, *p. neglectus* and *D. dipsaci* nematodes to find the source of resistance by different inoculation methods, experiment tube size, growth media, cultural technique and time of assessment.

4.1.1.1. Extraction time

Nine accessions of wild *Cicer* sp, 3 of *C. reteiculatum*, 3 of *C. echinospermum* and 3 line of *C. arietinum* were used to compare nematodes after 16 and 20 weeks. After harvest time, there was no significant difference at 16 weeks growing time and 20 weeks between chickpea species. However, harvesting after 16 weeks at the *C. echinospermum* species showed a lower number of nematodes in the *C. arietinum*. Accordingly, saving time is one of the most important noticeable signs of laboratory services, as more rapid results saves time and is common in the literature for harvesting time after 16 weeks to be more suitable than 20 weeks for the screening experiment (Figure 4.18).

Figure 4.1. The effect of extraction times of *P. thornei*, *P. neglechtus* and *Ditylenchus dipsaci* on chickpea species.



4.1.1.2. Inoculum density in growth chamber

The methodology data was analysed at Cukurova University. The complex 4-way experiment has condensed nicely into a simple 2-way interaction between species and density ($P < 0.087$), indicated that the main effects of, and interaction with an incubation period, variety within collection site within species were irrelevant (NS) (Table 6 and 7).

In the present study, the multiplication rate MR (P_f/P_i) techniques were the most commonly used genotypic identification of nematodes resistant. The P_f/P_i ratio was generally used in plant nematology as a measuring value to determine resistance. After injection a 150, 225 and 300 for root lesion and a 150, 300 and 400 inoculum density for *D. dipsaci* per plant, the MR was compared between 9 lines on 3 different chickpea species (Figure 4.19, 4.20, 4.21 and Table 5). This research had a limited number of accessions of wild chickpea species *C. reticulatum*, *C. echinospermum* and *C. arietinum* for lesion nematodes (*P. thornei* and *P. neglechtus*) to show the highest nematode reproduction factor in *C. arietinum*

species with inoculum density of 150 nematodes per plant were (MR=2.9) and the lowest reproduction nematodes at *C. echinospermum* species (MR=2.6) (Table 5). Also, similar studies in *D. dipsaci* indicated that the highest nematode reproduction factor in the *C. arietinum* with an inoculum density of 150 nematodes per plant was (MR=2.6) and the lowest population density was in the *C. echinospermum* of chickpea with MR= 2.3. This means that only at low population densities was there a difference at an inoculum density of 150 nematodes per plant between chickpea species in RLN and *D. dipsaci*. Also, result of analyses variance between three species with three different population densities of nematode were irrelevant (eg. not significantly different) and indicated that there was no greater significant difference between inoculum density of 225 and 300 nematodes per plant in root lesion nematodes ($p<0.013$) (Table 6) and inoculum density of 300 and 400 nematodes per plant in *D. dipsaci* ($p<0.011$) (Table 7). The difference was observed at a low population density of 150 nematodes per plant between species in root lesion nematodes and *D. dipsaci* $P<0.087$ (Table 6 and 7).

Final nematode of RLN (*P. thornei* and *P. neglectus*) counts rise to a peak at 225 nematodes per plant with no change as the population rises to 300 nematodes per plant in all species (Figure 4.19). Also, the final population density of *D. dipsaci* rise to a peak at 300 nematodes per plant there was no change indicated as the population rises to 400 nematodes per plant in all species (Figure 4.20 and 4.21). Therefore, inoculum density of 300 of *D. dipsaci* can be used for screening studies for all species. Moreover, there was some suggestion that *C. echinospermum* was more resistant to *P. thornei*, *P. neglectus* ($p<0.008$) (Table 6) and *D. dipsaci* ($p<0.05$) than *C. arietinum* and *C. reticulatum* (Table 7). The result of this study indicated that an inoculum density of 225 or 300 of RLN (*P. thornei* and *P. neglectus*) (Figure 4.19 and 4.21) and an inoculum density of 300 or 400 of *D. dipsaci* nematodes per plant with 16 weeks of growth was recommended for screening purposes (Figure 4.20). This was a good outcome for a methodology experiment as it suggests that common conditions can be used for all species.

The analysis of variance (ANOVA) was another way of measuring the difference between treatments is to calculate the variance between treatment means. The differences within treatment can be measured by calculating the average variance between final density within treatments. If the variance between treatments is much larger than the variance within treatments then there is a difference between treatments (Table 6 and 7).

Figure 4.2. The effect of initial population density of *P. thornei* and *P. neglectus* on 3 Cicer species incubated with 3 population densities.

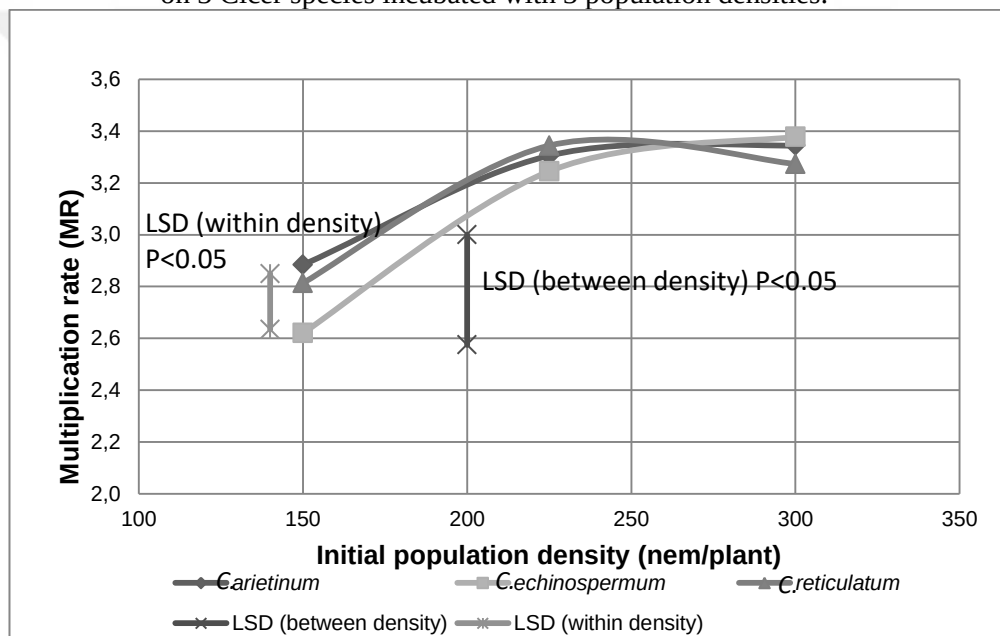


Figure 4.3. The effect of initial population density of *D. dipsaci* on 3 chickpea species.

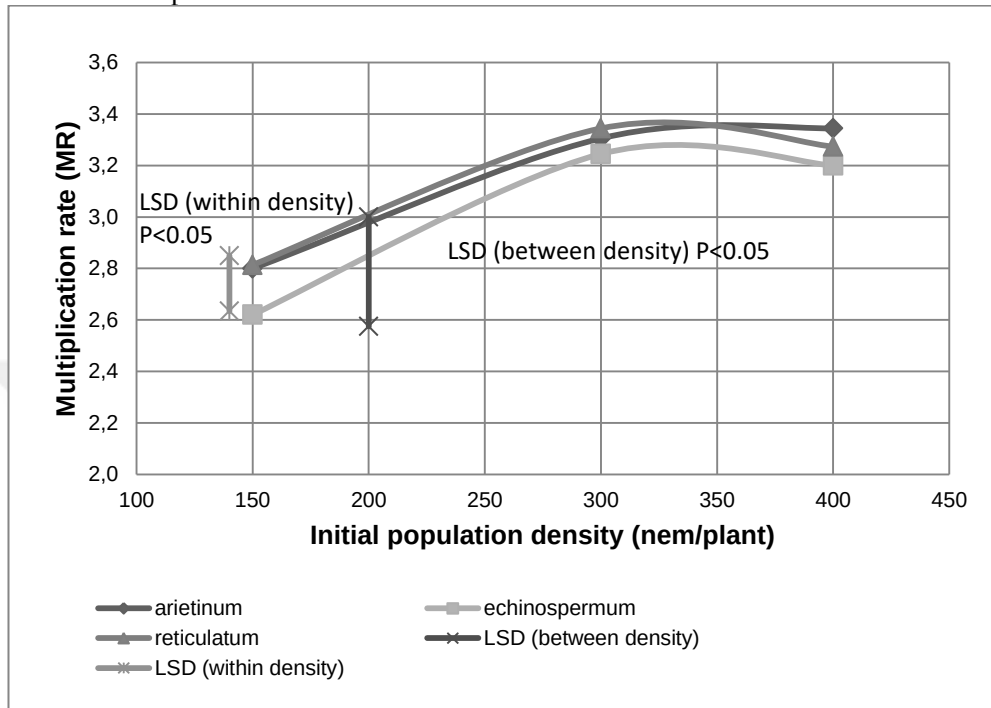
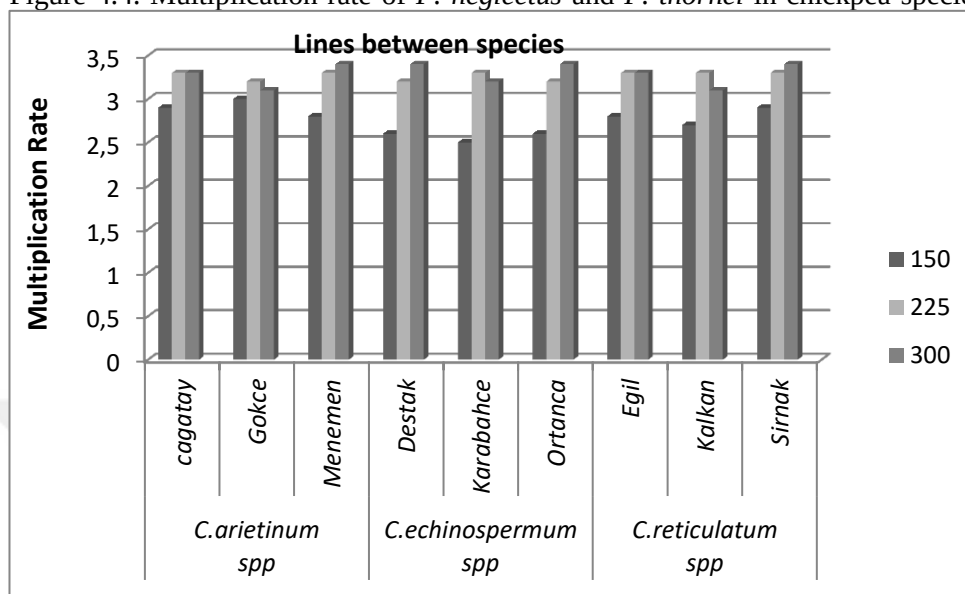


Table 5. The effect initial population density on the multiplication rate (MR) of nematodes between 3 different species and lines of chickpea.

Parametre	Group	<i>Cicer arietinum</i> spp			<i>Cicer echinospermum</i> spp			<i>Cicer reticulatum</i> spp		
		Çagatay	Gökçe	Menemen	Destak	Karabahçe	Ortanca	Egil	Kalkan	Sırnak
Multiplication rate of <i>P. neglectus</i> and <i>P. thornei</i>	16 weeks, 150 nematodes	2.9	2.9	2.8	2.6	2.5	2.6	2.8	2.7	2.8
	20 weeks, 150 nematodes	2.9	2.8	2.8	2.7	2.6	2.7	2.8	2.7	2.8
	16 weeks, 225 nematodes	3.3	3.2	3.3	3.2	3.1	3.2	3.3	3.2	3.3
	20 weeks, 225 nematodes	3.2	3.3	3.3	3.3	3.1	3.3	3.3	3.2	3.2
	16 weeks, 300 nematodes	3.3	3.1	3.2	3.4	3.2	3.4	3.3	3.1	3.3
	20 weeks, 300 nematodes	3.3	3.2	3.3	3.4	3.2	3.3	3.3	3.2	3.3

LSD (between density): 0.4 LSD (within density): 0.2

Figure 4.4. Multiplication rate of *P. neglectus* and *P. thornei* in chickpea species.Table 6. Summary effect of initial population density of *P. neglectus* and *P. thornei* responses to *C. echinospermum*, *C. reticulatum* and *C. arietinum*.

Source of variation	<i>d.f.</i>	<i>s.s.</i>	<i>m.s.</i>	<i>v.r.</i>	<i>F pr.</i>
Density	2	14.1543	7.0771	5.62	0.013
Species	1	11.2589	11.2589	8.94	0.008
Interaction density within Species	2	0.7065	0.3533	2.48	0.087
Residual	144	694.,390	1.159	-	-
Total	215	3.433.861	-	-	-

Table 7. Summary effect of initial population density of *D. dipsaci* responses to *C. echinospermum*, *C. reticulatum* and *C. arietinum*.

Source of variation	<i>d.f.</i>	<i>s.s.</i>	<i>m.s.</i>	<i>v.r.</i>	<i>F pr.</i>
Density	2	14.1543	7.0771	5.62	0.011
Species	1	11.2589	11.2589	8.94	0.005
Interaction density within species	2	0.7065	0.3533	2.48	0.087
Residual	144	694.390	1.159	-	-
Total	215	3.433.861	-	-	-

Means square (m.s): the variance between treatments is shown in the Mean Square column next to density, and the variance within treatments, labelled as Error, is shown below it.

F ratio: the ratio between the treatment variance and is within treatment variance is shown in the F column next to density. This value (5.62), called the F ratio, is much greater than 1, indicating that the between treatment variance is much greater than the within treatment variance.

P values: next to the F ratio is the probability that this F ratio could occur if the treatments were the same. In this case the probability of an F ratio of 5.62 occurring when the treatments are the same is 0.000 or very unlikely. Therefore, we say that the treatments are highly likely to be different.

There are a number of other items of interest in the analysis of variance table above. The column headed contains degrees of freedom which are a measure of how much information is contained in each variance. A variance between two means, such as the density mean square, contains 2 degrees of freedom (two species of nematodes *P. thornei* and *P. neglectus*) after correction for the overall mean. The column headed *s.s.* contains the sums of squares, which are calculated by multiplying the mean square and degrees of freedom in the same row. Notice that the degrees of freedom and the sums of squares for density and residual add to the corresponding values for total.

4.1.2. Effect of temperature, time and inoculum density on reproduction of *Ditylenchus dipsaci* in carrot disk culture.

There were no further studies regarding the culture of *D. dipsaci* on carrot disks and no further references reported about factors such as the effect of time, temperature, and inoculum density on nematode reproduction.

4.1.2.1. The effect of time of incubation period on population density of *Ditylenchus dipsaci*

In experiment 1, at 19°C, the population density of nematode and reproduction rate of *D. dipsaci* increased with increasing time after inoculation (Table 8). Twenty days after inoculation, about 50 female and males were placed per disinfected surface of the initial inoculum.

Andrejev (1975) reported that a week after the last moult, copulation was observed when the female becomes receptive to receiving sperms and the reproduction of *D. dipsaci* is emphatic. The moult from the juvenile in the first stage (J1) to J2 is different and depends on the temperature and time period. Andrejive (1977), cited in Gubina, (1988) reported that “The moult from the juvenile in the first stage (J1) to J2, takes 60 to 55 at 1° to 3°c or about 6 days at 21°c to 25°c.” Clearly, the first life cycle was not completed, as the indicated number of females at 20 days was close to initial inoculum density. Similarly, 40 days after inoculation, the nematode population density was a mixture of the first and second generation. At this time, the total number of females was close to the number of juveniles 25 days earlier.

Therefore, it seems that at low population density the generation time was between 20 and 40 days, and after 40 days indicated that there was about four-fold increase per generation inoculation. By 45 days after inoculation, the total number were beginning to increase and be more than three times the total number of nematodes 20 days earlier. Experiment 1 indicated that the reproduction rate of *D. dipsaci* in carrot disks were 600 after 45-60 days, compared to 6.3 after 20 and

MR= 59.2 after 40 that were recorded. Also, experiment 1 indicated that a higher number of nematodes and reproduction rate occurred at 45 and 60 days than 20 and 40 days and there were no differences observed between 60 and 90 or 45 and 60 days (Table 8). Each carrot disk was inoculated with an initial population density (P_i) of 50 females and males and incubated at $19 \pm 1^\circ\text{C}$.

4.1.2.2. The Effect of temperature on nematode reproduction

In experiment 2, higher numbers of *D. dipsaci* nematodes and reproduction rate occurred between 19°C and 25°C than 15°C and 30°C and 30°C (Table 9) and there were no further significant differences between 20°C and 25°C or between 15°C and 30°C .

The optimum temperature for reproduction of *D. dipsaci* was at 20°C and 25°C . The temperature range can affect reproduction and development of nematodes also, as Seinhorst (1950) reported that between 5°C and 26°C there was no temperature effect on the activity of *D. dipsaci*. Similarly, studies indicated the optimum temperature for development is between 15°C and 20°C and the minimal activity of this nematode is at 5°C .

Croll (1967) and Griffin (1974; 1987) reported that the activity of *D. dipsaci* was identified by the temperature regime to which nematodes were previously exposed. Also, for reproduction and development of *D. dipsaci* nematode, optimum activity has been reported as (Sayre and mountain, 1962) and 20°C for alfalfa (Griffin, 1968). However, the varying fecundity of *D. dipsaci* females can be primarily a response to temperature or other environmental factors. This study was undertaken to assess the effect of temperature on nematode growth reproduction rate of *D. dipsaci* using carrot disks to understand which temperature factors have influenced reproduction rate of the *D. dipsaci* nematode. In this current study, the number of females and the development rate between 20°C and 25°C at 45-60 days after inoculation was nearly 4 time the numbers after 20 days at 25°C (Table 8 and 9). Therefore, the nematodes completed its life cycle in about 25- 35 days at $20\text{-}25^\circ\text{C}$.

This result can provide a useful observation for studies on the biology of *D. dipsaci*. Each carrot disk was inoculated with an initial population density (P_i) of 50 females and nematodes and were extracted 35 days after inoculation.

4.1.2.3. The effect of initial inoculum density on nematode reproduction

Experiment 3, (Table 10) indicated that the population density of nematodes increased after 45 days at 25°C with increasing inoculum density (Figure 5). The highest reproduction rate occurred with an average inoculum of 100 *D. dipsaci* per disk and reduction of nematode reproduction rate was observed for *D. dipsaci* under greenhouse conditions. Also, it was found that with increasing nematode inoculum density, the reproduction rate of *D. dipsaci* was reduced for chickpea plant. Studies have reported similarly for lesion nematodes (*P. thornei*) on chickpea under laboratory conditions (Castilo et al., 1994).

This reduction of nematode reproduction could be explained as a result of competition for food resources. As result of this study, it is indicated that maximum yields of activity and available *D. dipsaci* that carrot disk cultures can obtain after 45 days of incubation at 20°C and the optimum initial inoculum density for reproduction rate was 100 nematodes per disk at 45 days after inoculation and incubation at 20°C. Each carrot disk was inoculated with an inoculum density (P_i) of either 50, 100, 200, 300, and 500 *D. dipsaci* with mixed life stages and incubated at $19 \pm 1^\circ\text{C}$. Also, nematodes were extracted 35 days after incubation.

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Table 8. Reproduction of *Ditylenchus dipsaci* on carrot disks after four time periods.

The number of nematodes per carrot disk					
Time (Day)	Female	Juvenile	Eggs	PF	MR
20	67	220	30	370	7.4
40	860	1,200	100	2,960	59.2
60	4,800	1,300	12,200	30,000	600
90	5,200	15,500	11,500	32,200	644
LSD _{0.05}	1,353.1	5,645.5	4,127.2	11,126	222.5

Pf = Final population; MR (Multiplication rate) = Pf/Pi.

Table 9. Reproduction of *Ditylenchus dipsaci* on carrot disks at different temperatures.

The number of nematodes per carrot disks					
Temperature °C	Female	Juvenile	Eggs	PF	MR
15	15	9	30	54	1.08
20	68	120	80	268	5.360
25	60	92	72	224	4.48
30	8	4	3	15	0.3
LSD _{0.05}	12.45	35.7	22.8	70.95	1.41

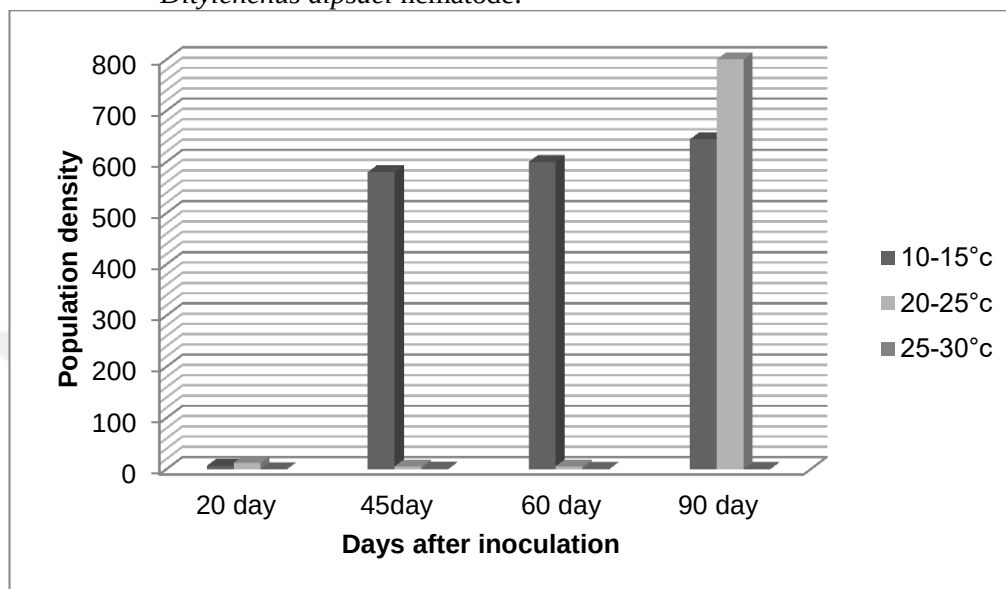
Pf = Final population; MR (Multiplication rate) = Pf/Pi.

Table 10. The effect of inoculum density on reproduction of *Ditylenchus dipsaci* on carrot disks.

The number of nematodes per carrot disks					
No. of inoculum density	Female	Juvenile	Eggs	PF	MR
50	137	260	290	687	13.74
100	240	1,580	960	2,780	27.8
200	540	2,200	1,880	4,620	23.1
300	885	3,200	2,200	6,285	21.6
500	1,100	5,100	3,420	9,620	19.24
LSD _{0.05}	390,000	1,902	880	3,172	4.26

Pf = Final population; MR (Multiplication rate) = Pf/ Pi

Figure 4.5. The effect of both factors, time (20, 45, 60 and 90 days) and temperature (10, 15, 20, 25 and 30°C) on population density of *Ditylenchus dipsaci* nematode.



4.1.3. Evaluation of wild *cicer* species (*C. reticulatum* and *C. echinospermum*) against to *P. thornei*, *P. neglectus* and *D. dipsaci* for resistance.

Conducted of two experiment accession of wild *Cicer* spp. Against three nematodes for resistance including; Experiment 1 and 2. Experiment 1, evaluated population density increase of *P. thornei* and *P. neglectus* in *Cicer arietinum* (n=3), *C. echinospermum* (n=82) from 8 collection sites and *C. reticulatum* (n=211) from 24 collection sites and Experiment 2 evaluated population density increase of *D. dipsaci* in *C. arietinum* (n=3), *C. echinospermum* (n=42) from 4 collection sites and *C. reticulatum* (n=108) from 6 collection sites. Analyses variate (Final population density) between treatment variance of *D. dipsaci*, *P. thornei*, and *P. neglectus* indicated that there was a significant difference ($P < 0.001$) between chickpea species, nematode species, nematode species within *Cicer* Species and Nematode sp within *Cicer* sp and Collection site in this study (Table 11 and 14).

Great care and caution is needed to make accurate inferences about differences between and within species, collection sites, and accessions when examining ANOVA from experiments 1 and 2. Nested ANOVA indicates that both experiments 1 and 2 returned highly significant differences at all levels of organization: species, collection sites within species and accessions within collection sites within species (Table 11 and 14).

4.1.3.1. Experiment 1

The responses to *P. thornei* and *P. neglectus* were dominated by species differences between *C. echinospermum* and *C. reticulatum* that became even more important in Experiment 1, where these comparisons are more balanced. In both experiments, *C. arietinum* was more susceptible than *C. reticulatum* and *C. echinospermum*. Note that this was at odds with the consistent results coming from the field result study of nematode population dynamics. However, because there was significant remaining variation between collection sites within species and between accessions within collection sites within species for responses to these nematodes, it was conceivable that the much smaller field studies simply reflect this variation.

4.1.3.1. (1). *Pratylenchus neglectus*

This research conducted with 296 numbered accessions of wild chickpea species, 211 of *C. reticulatum* and 82 of *C. echinospermum* for resistance against *pratylenchus neglectus* showed that, 21 accessions of *C. reticulatum* out of 211 including Ak: 202, 463, 465, 468, 472, 473, 489, 494, 528, 529, 531, 538, 543, 574, 577, 648, 649, 687, 715, 716, 689; and 11 accessions of *C. echinospermum* out of 82, Ak: 232, 434, 454, 457, 595, 601, 609, 612, 616, 620, 621 were rated < 1 and were accepted resistant to *P. neglectus* (Table 13). Also, population fluctuation of the two species of chickpea species showed that there was significant difference

between *C. echinospermum* and *C. reticulatum* to *P. neglectus* (Table 17 and Figure 4.23).

4.1.3.1. (2). *Pratylenchus thornei*

Within 296 accessions of two different wild Cicer species, 211 of *Cicer reticulatum* and 82 of *C. echinospermum* were tested against *P. thornei* for resistance. As a result, the study indicated, that 14 accessions of *C. reticulatum* out of 211 including, Ak:183, 494, 497, 516, 580, 648, 649, 651, 652, 688, 689, 719, 720 and 5 accessions of *C. echinospermum* species out of 82 including Ak: 82, 161, 430, 433, 434 were rated ≤ 1 and were considered resistant to *P. thornei*. Also, the population variation of the two chickpea species showed that there was a significant difference between *C. echinospermum* and *C. reticulatum* to the *P. thornei* (Table 17 and Figure 4.24).

4.1.3.1. (3). Results of study in experiment 1 indicated that

- ✓ *Cicer reticulatum* showed 8-fold differences between selection sites (Table 11 and 12).
- ✓ A very strong *Cicer* by nematode sp interaction ($P < 0.001$) (Table 11 and Figure 4.25).
- ✓ Wild Chickpeas were more resistant to nematodes than domestic (Table 11 and 12).
- ✓ *Cicer arietinum* and *C. reticulatum* were more resistant to *P. neglectus* than *C. echinospermum*. However, *C. reticulatum* was far more resistant than *C. arietinum* (Table 10, 11, 12 and Figure 4.23, 4.24 and 4.25).
- ✓ *Cicer echinospermum* was more resistant to *P. thornei* than *C. arietinum* and *C. reticulatum*. However, *C. reticulatum* was far more resistant than *C. arietinum* ($P < 0.001$) (Table 10 and Figure 4.24 and 4.25).

- ✓ Even though *C. echinospermum* was more susceptible to *P. neglectus* than to *P. thornei*, it was more resistant than the domestic *C. arietinum* $P < 0.001$ (Table 11, 12 and 13).
- ✓ Differences between collection sites within species are larger than between accessions within collection sites. Nevertheless, these smaller, albeit significant 4-way interactions, suggest that there were important differences between accessions within species (Table 11).

Table 11. Analysis of variance: *Pratylenchus thornei* and *P. neglectus*

Source of variation	d.f.	s.s	m.s	Var ratio	P val
Rep stratum	3	137,468	45,823	4.22	
Rep.Nem_sp stratum					
Nem_sp	1	1,317,901	1,317,901.	121.29	0.002
Residual	3	32,597.	10,866.	2.66	
Rep.Nem_sp.*Units* stratum					
Sp	2	2,404,845	1,202,422.	294.83	<.001
Nem_sp.Sp	2	8,327,146	4,163,573	1,020.88	<.001
Sp.Coll_site_code	32	7,871,127.	245,973	60.31	<.001
Nem_sp.Sp.Coll_site_code	32	1,803,699.	56,366.	13.82	<.001
Sp.Coll_site_code.AK_Line	261	14,154,652.	54,232.	13.30	<.001
Nem_sp.Sp.Coll_site_code.AK_Line	261	4,959,697	0.19165	4.66	<.001
Residual	1769	7,214,726	4,078.		
Total	2,366	47,987,933			

Var ratio: the ratio of the **between treatment** variance to the **within treatment** variance is shown in the V. ratio column next P value. This value, called the F ratio, is much greater than 1, indicating that the between treatment variance is much greater than the within treatment variance.

P values: next to the F ratio is the probability that this F ratio could occur if the treatments were the same. In this case the probability of an F ratio occurring when the treatments are the same is 0.000 or very unlikely. Therefore, we say that the treatments are probably different or *significantly* different. The probability is called the significance.

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Table 12. The means of *P. thornei* and *P. neglectus* on chickpea species.

Nematode species	<i>P. neglectus</i>	<i>P. thornei</i>		
	274.8a	322.0b		
LSD	13.63			
Chickpea Species	<i>C. arietinum</i>	<i>C. echinospermum</i>	<i>C. reticulatum</i>	
	602.5a	308.7b	290.1c	
LSD	25.75			
Nematode species	Chickpea species	<i>C. arietinum</i>	<i>C. echinospermum</i>	<i>C. reticulatum</i>
<i>P. neglectus</i>		505.0a	380.7b	230.4c
<i>P. thornei</i>		700.0a	236.6b	349.8c
LSD	44.88			

Table 13. Least significant differences of means (5% level)

Table	Nem_sp	Sp	Nem_sp	Sp	
rep.	1,184	unequal	unequal	unequal	
l.s.d.		36.16X	51.58	62.63	min.rep
d.f.		1769	1297.51	1769	
l.s.d.	13.63	25.75	37.05	44.88	max-min
d.f.	3	1769	717.49	1769	
l.s.d.		4.31X	12.79	10.30X	max.rep
d.f.		1769	3.98	1769	
Except when comparing means with the same level(s) of					
Nem_sp			51.13		min.rep
d.f.			1769		
			36.41		max-min

(No comparisons in categories where l.s.d. marked with an X)

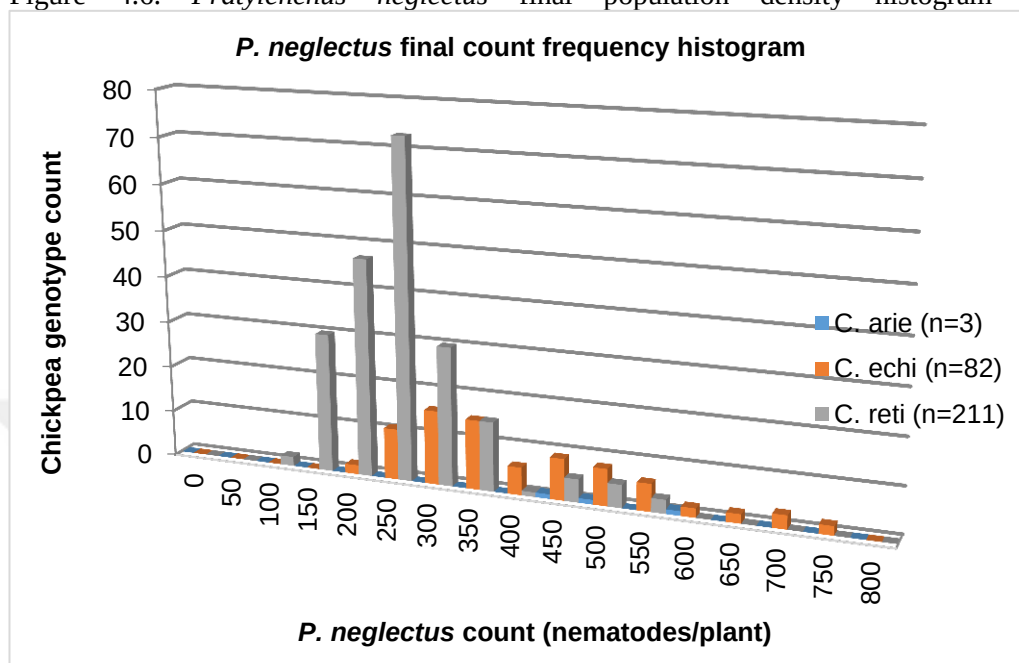
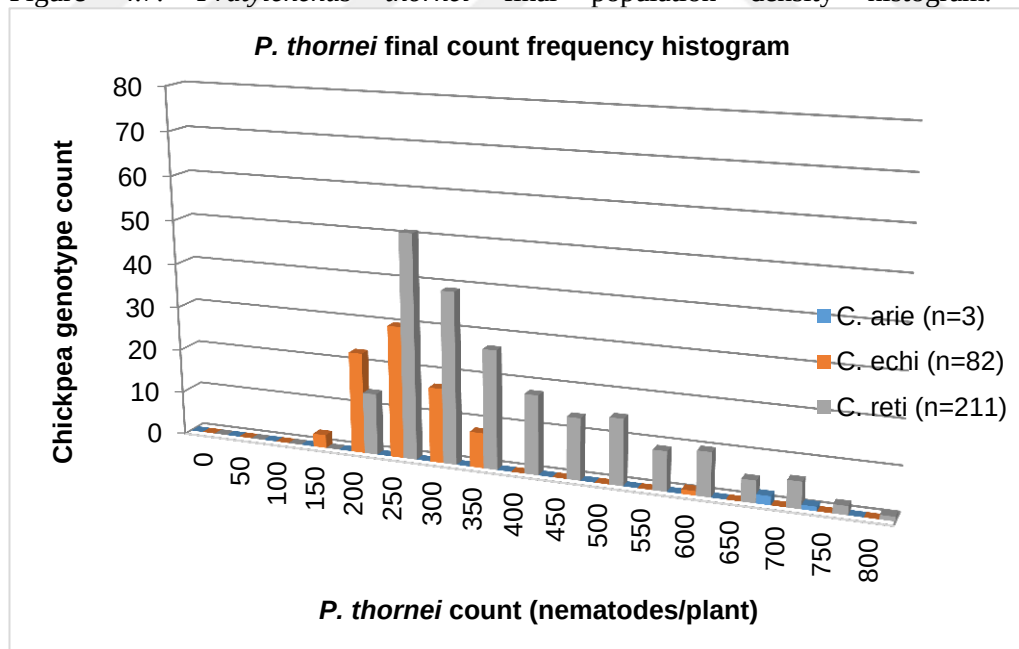
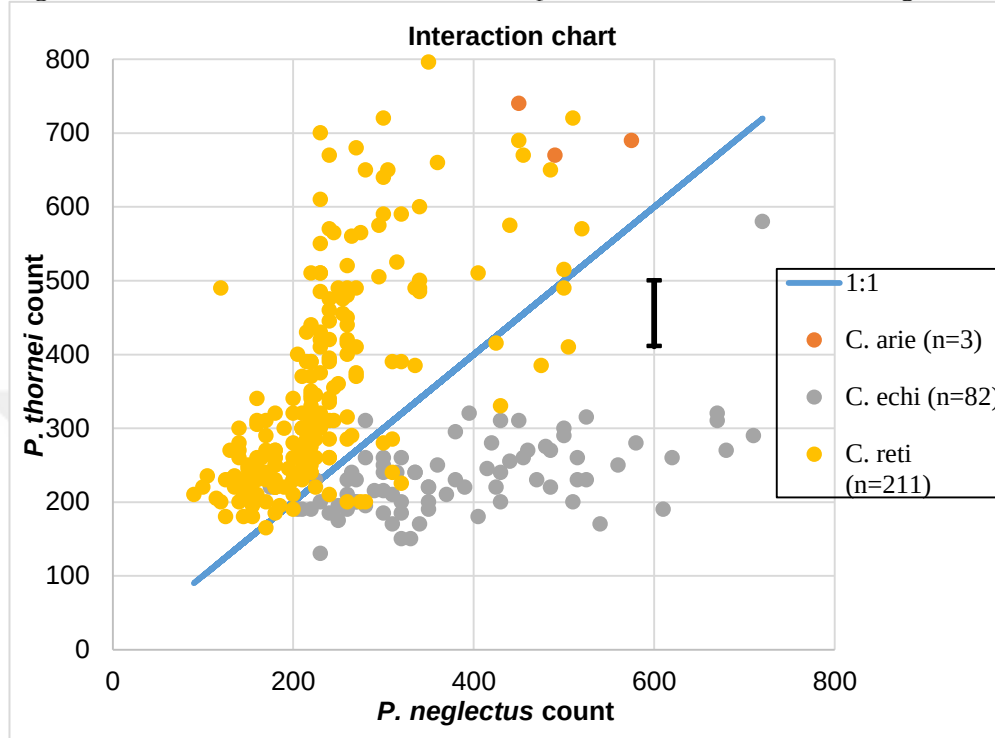
Figure 4.6. *Pratylenchus neglectus* final population density histogramFigure 4.7. *Pratylenchus thornei* final population density histogram.

Figure 4.8. Interaction chart between *Cicer* species and *P. thornei* and *P. neglectus*.

4.1.3.1. (4). The histogram of residual for *P. thornei* and *P. neglectus*

The simplest model for this experiment was a one-way ANOVA by using a histogram of residuals to assess whether residuals were normal or half normal by plot distribution in experiment 1 (Figure 4.23 and 4.25). Standardized residuals for all predicted values should be distributed symmetrically. The histogram of residuals must be bell-shaped, like a normal distribution curve and both the normal and half normal plot should be straight lines at 45°. In this instance the residual plot was satisfactory, although, the residual histogram was somewhat skewed to the right, and as a result, there was some deviation from linear in both normal plots. Because of this, there was a reflection of the experimental design (Figure 4.26 and 4.27).

Figure 4.9. The simplest model for this experiment is a one-way ANOVA:

***** Analysis of variance *****

Variate: Final count of nematode (*Pratylenchus neglectus*)

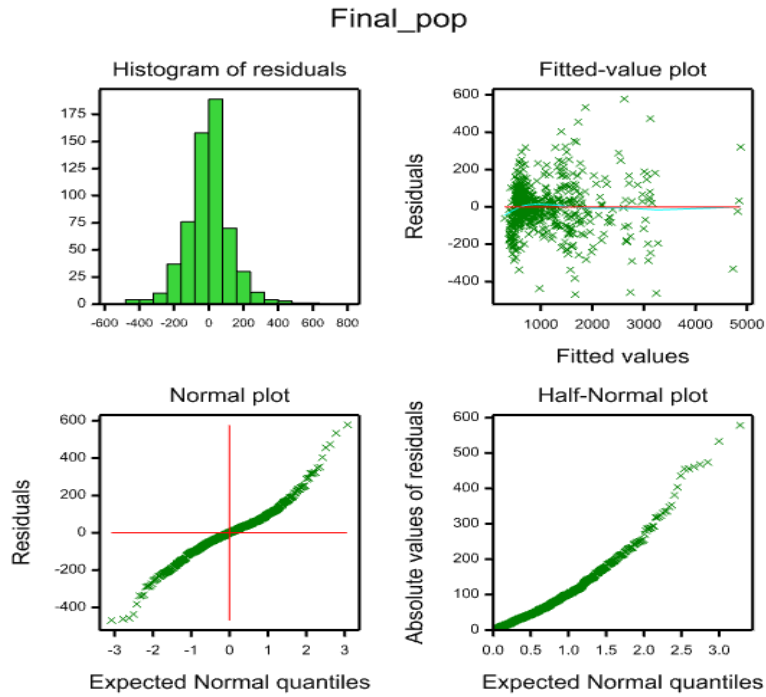
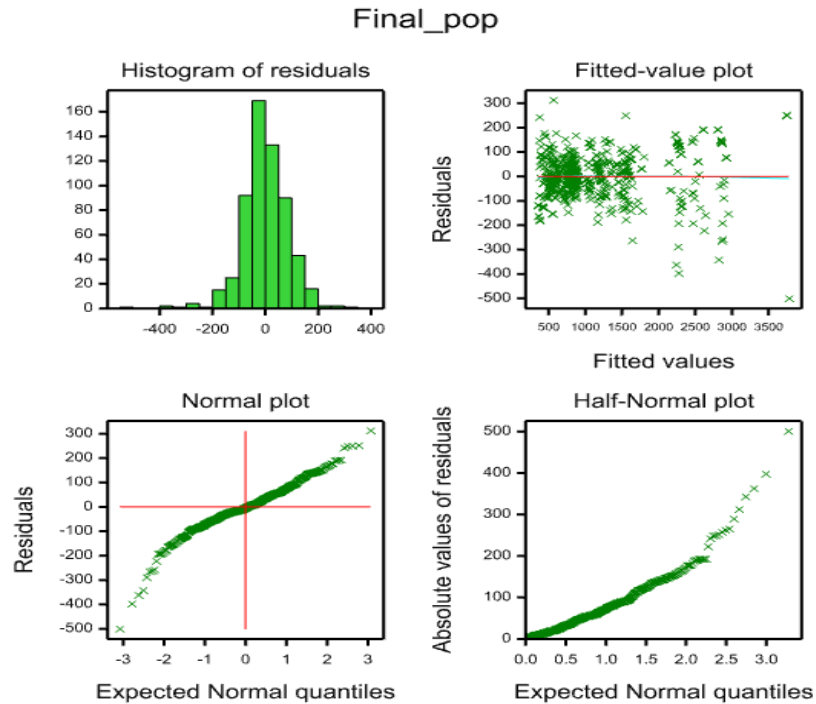


Figure 4.10. The simplest model for this experiment is a one-way ANOVA:

***** Analysis of variance *****

Variate: Final count of nematode (*Pratylenchus thornei*).



4.1.3.1.3. (2). The means of ANOVA for *P. thornei* and *p. neglectus*

The histogram of residual and fan-shaped experiments above indicated that there was a significant difference between variance in this experiment. In addition, the residuals were normally distributed as indicated by the skewed histogram, and deviated from linearity in the normal plots. The histogram of residual was much less skewed and the normal plots look much more like straight lines. Also, ANOVA indicates that there was significant treatment of differences in the trial ($P < 0.001$). Moreover, the implicit assumption is that nematodes populations were evenly distributed in the trial and that any differences in final counts were due to experimental treatments only.

4.1.3.2. Experiment 2**4.1.3.2. (1). *Ditylenchus dipsaci***

The observation of roots and soil indicated that most of the Chickpea lines were affected by the *D. dipsaci* and there were significant differences between lines of *C. echinospermum* and *C. reticulatum*. A total of 150 accessions of wild *Cicer* sp were conducted to *D. dipsaci*. Thirteen lines of *C. reticulatum* out of 108 including: 3, 7, 9, 11, 39, 43, 47, 50, 55, 58, 67, 100, 102 and 12 accessions of *Cicer echinospermum* out of 42 including: Ak: 75, 79, 80, 82, 161, 163, 164, 165, 166, 168, 170, 174 were rated ≤ 1 and considered resistant to *D. dipsaci* and 13 lines of *C. echinospermum* out of 42 were rated ≤ 1 and resistant to *D. dipsaci* (Table 17). The responses to *D. dipsaci* were dominated by species differences between *C. echinospermum* and *C. reticulatum* which became even more important in Experiment 2, where these comparisons were more balanced. There was a significant difference between chickpea species, collection sites within chickpea species and between accessions within collection sites within species for responses to these nematodes (Table 14, 15 and Figure 4.27). It was conceivable that the much smaller field studies simply reflect this variation. In Experiment 2 there were 2-fold differences among *C. echinospermum* collection sites and 5-fold differences among *C. reticulatum* collection sites (Table 14 and Figure 4.27).

4.1.3.2. (2). The Results of Experiment 2 Indicated that

- ✓ There was very strong *Cicer* and nematode species interaction ($P < 0.001$) (Table 14, 15 and Figure 4.27)
- ✓ Wild chickpea were more resistant to *D. dipsaci* than domestics (Table 14 and Figure 4.27).

- ✓ *Cicer echinospermum* was more resistant to *D. dipsaci* than *C. arietinum* and *C. reticulatum*. However, *C. reticulatum* was far more resistant than *C. arietinum* ($P < 0.001$) (Table 14, 15 and Figure 4.27).
- ✓ Differences between collection sites within species were larger than between accessions within collection sites. This suggests that there were important differences between accessions within species (Table 14 and 15).

Table 14. Analysis of variance for *Ditylenchus dipsaci*

Source of variation	d.f.	s.s	m.s	Var ratio	P value
Rep stratum	3	185,354.	61,785.	12.87	
Rep.Nem_sp.*Units* stratum					
Sp	1	173,993.	173,993	36.26	<.001
Nem_sp.Sp	2	602,537.	602,537.	125.56	<.001
Sp.Coll_site_code	11	23,555,677.	2,141,425	446.23	<.001
Nem_sp.Sp.Coll_site_code	11	5,400,201.	490,702.	102.25	0.010
Sp.Coll_site_code.AK_Line	137	42,380,777.	395,120.	82.34	<.001
Nem_sp.Sp.Coll_site_code.AK_Line	137	14,861,071.	138,444.	28.851	<.001
Residual	298	2,135,500.	4,799.		
Total	597	78,032,886.			

Var ratio: The ratio of the **between treatment** variance to the **within treatment** variance is shown in the V. ratio column next P value. This value, called the F ratio, is much greater than 1, indicating that the between treatment variance is much greater than the within treatment variance.

P values: Next to the F ratio is the probability that this F ratio could occur if the treatments were the same. In this case the probability of an F ratio occurring when the treatments are the same is 0.000 or very unlikely. Therefore, we say that the treatments are probably different or *significantly* different. The probability is called the significance.

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Table 15. The means of *Ditylenchus dipsaci* on chickpea species.

Chickpea Species	<i>C. arietinum</i>	<i>C. echinospermum</i>	<i>C. reticulatum</i>
	702.5a	877.2b	839.3c
LSD	26.15		

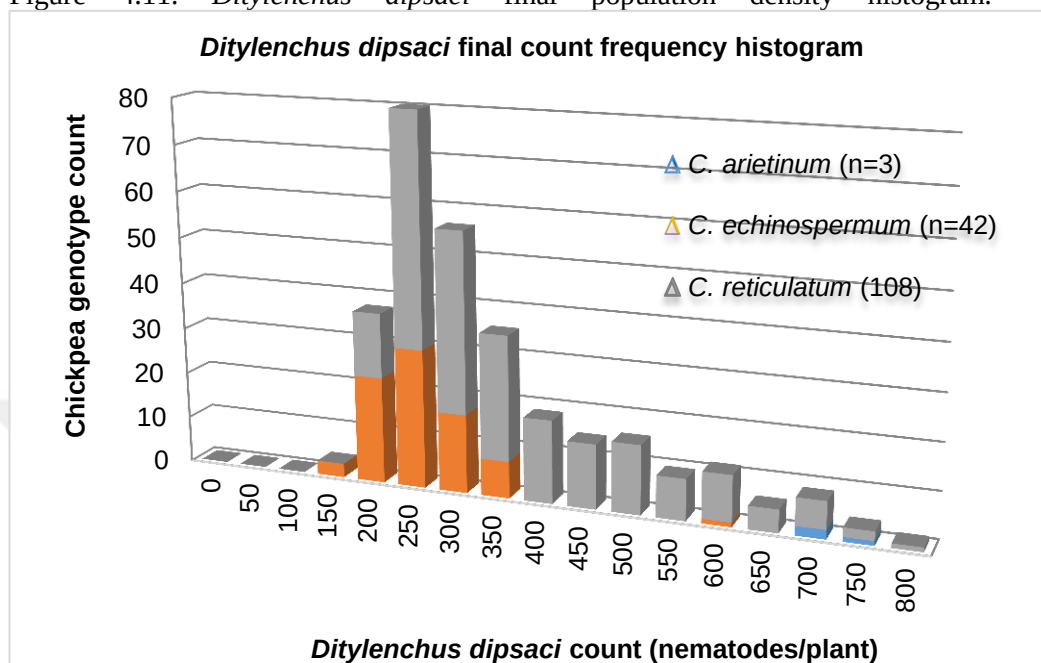
- Wild chickpea is more resistant to *D. dipsaci* than domestic (*C. arietinum*)

Nematode species	Chickpea species	<i>C. arietinum</i>	<i>C. echinospermum</i>	<i>C. reticulatum</i>
<i>D. dipsaci</i>		863.7a	595.0b	750.0c
LSD	146.78			

Table 16. Least significant differences of means (5% level)

Table	Sp	Nem_sp Sp	Sp Coll_site_code	
rep.	unequal	unequal	4	
d.f.	445	445	445	
l.s.d.		203.39X		min.rep
	26.15	146.78	203.39	max-min
		41.52X		max.rep

(No comparisons in categories where l.s.d. marked with an X)

Figure 4.11. *Ditylenchus dipsaci* final population density histogram.

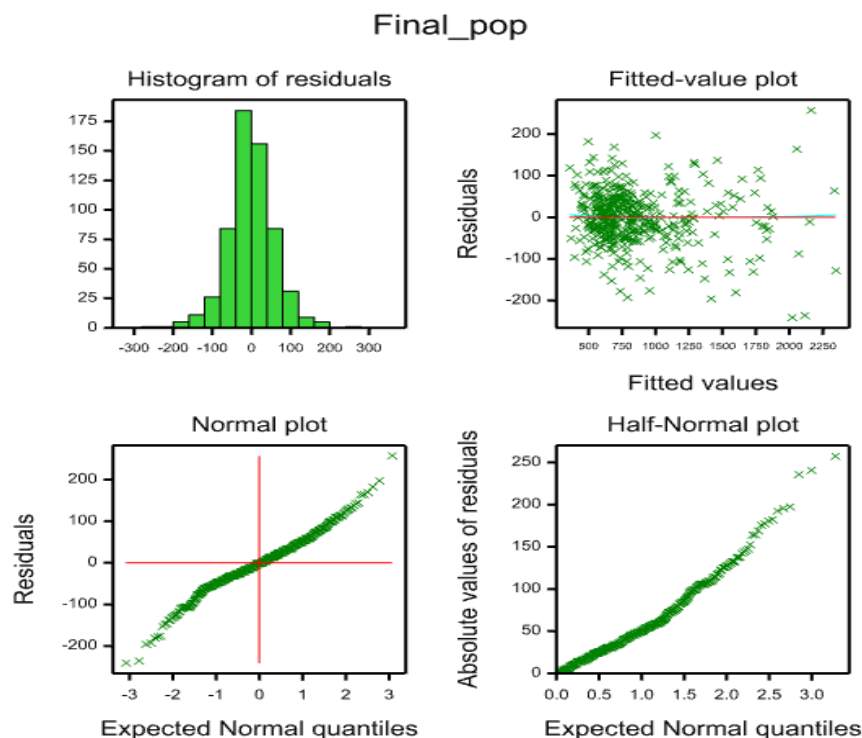
4.1.3.2. (3). The histogram of residuals for *Ditylenchus dipsaci*

The histogram of residuals was used to assess whether residuals had a normal or half normal plot distribution in experiment 2 (Figure 12). Standardized residuals for all predicted values should be distributed symmetrically. The histogram of residuals must be bell-shaped, like a normal distribution curve and both the normal and half normal plot should be straight lines at 45°. In this instance the residual plot was satisfactory, although, the residual histogram was somewhat skewed to the right, and as a result, there was some deviation from linear in both normal plots. Because of that, there was a reflection of the experimental design (Figure 4.28).

Figure 4.12. The simplest model for this experiment is a one-way ANOVA:

***** Analysis of variance *****

Variate: Final count of nematode (*Ditylenchus dipsaci*)



4.1.3.2.3. (1). The means of ANOVA for *Ditylenchus dipsaci*

The histogram of a residual and fan-shaped experiment for *D. dipsaci* above confirmed that there was a significant difference between variants in this experiment. In addition, the residuals were normally distributed as indicated by the skewed histogram, and deviated from linearity in the normal plots. The histogram of residual was much less skewed and the normal plots look much more like straight lines. Also, ANOVA indicates that there was significant treatment of differences in the trial ($P < 0.001$).

Table 17. The list of result of consistently multiple resistant and susceptible accession of wild *Cicer* species (*C. reticulatum* and *C. echinospermum*) against to *P. thornei*, *P. neglectus* and *D. dipsaci* under cabinet experiments.

Nematode	Resistant genotype	Susceptible genotype
<i>C. reticulatum</i>		
<i>P. thornei</i>	Ak:183, 494, 497, 516, 564, 580, 648, 649, 651, 652, 688, 689, 719, 720.	AK: 690, 470, 478, 488, 495, 533, 534, 550, 686.
<i>P. neglectus</i>	Ak:202, 463, 465, 468, 472, 473, 489, 494, 528, 529, 531, 538, 543, 574, 577, 648, 649, 687, 715, 716, 689.	AK: 478, 504, 668, 670, 679, 681, 685, 686.
<i>D. dipsaci</i>	Ak:3,7,9,11,39,43,47,50,55,58,67,100,102.	No significant difference
<i>C. echinospermum</i>		
<i>P. thornei</i>	Ak:82,161,430,433,434	AK: 443, 444, 449, 596, 599, 611, 620, 623, 625.
<i>P. neglectus</i>	Ak:232, 434, 454, 457, 595, 601, 609, 612, 616, 620, 621.	AK: 73, 75, 78, 79, 161, 163, 164, 165, 168, 169, 170, 172, 174, 175, 176.
<i>D. dipsaci</i>	Ak:75, 79, 80, 82, 161, 163, 164, 165, 166, 168, 170, 174.	AK: 73, 78, 169, 172, 175, 176.

*Resistance accession MR <1.

*Susceptible accession MR ≥1.

*Ak: 434, 494, 648, 649, 689 were as resistant to both root lesion nematodes (*P. thornei* and *P. neglectus*) as any *C. echinospermum* and *C. reticulatum* species. Also, Ak: 428, 443, 449, 686 as any *C. reticulatum* and *C. echinospermum* species indicated the same result to both root lesion nematodes (*P. thornei* and *P. neglectus*) and were susceptible to these nematodes.

4.2. Field study

4.2.1. Plant-parasitic nematodes associated with chickpea

The results of field study indicated that root lesion nematodes (*P. thornei*, *P. neglectus*) and *D. dipsaci* caused major damage to legumes crops like Chickpea. Moreover, Chickpea was one of the most susceptible crops that showing to be the good host of *P. thornei*, *P. neglectus* and *D. dipsaci* in Turkey. In this survey study, a total of 211 samples from 17 chickpea growing regions indicated that *P. thornei* and *P. neglectus* were the predominant plant-parasitic nematodes in the chickpea growing region in Turkey, in 2014 and 2016.

4.2.1.1. Plant parasites of legumes

The list of distribution by percentage of *P. thornei*, *P. neglectus* and *D. dipsaci* is presented in different regions of Turkey (Table 18, 20 and Figure 4.29). According to the results observed, the distribution of root lesion nematodes specially *P. thornei* was greater than other plant parasitic nematodes (Table 18). Also, *P. neglectus* and *D. dipsaci* were found respectively to be the most impactful plant parasitic nematodes after *P. thornei* in all sampling sites in Turkey (Figure 4.29).

4.2.1.2. Symptoms and percentage distribution of important nematodes in Chickpea.

The result of study indicated that root lesion nematodes (*P. thornei* and *P. neglectus*) were widely distributed in growing chickpea field in Turkey (Table 18). They were in 88% of the chickpea samples collected, and very evident in the infested plants. The symptoms of these nematodes such as stem and leaf necrosis and pod distortion were observed. Also, the plants roots were often becoming completely necrotic, black, showing poor and yellowing in some part of the plant. Almost one hundred of 211 samples were infected with a single species

of root lesion nematodes *P. thornei* which is very common in Turkey and it was detected in (84%) of the sample.

The geographical distribution of the most important plant-parasitic nematodes in chickpea growing fields is showed (Figure 4.29). Also, this study indicated that the other most common species after *P. thornei* were *P. neglectus* (65%), *P. penetrans* (58%) and *D. dipsaci* (khuen) Filipjev was extracted of 45% soil sample (Table 18 and Figure 4.29).

Other nematode analysis of soil samples among of several other plant parasitic nematodes were species of *Helicotylenchus* (50%), *Paratylenchoides*, *Trophurus* (4%), *Criconemoides* (1%), *Tylenchus* (47%), *Xhiphinema* (5%), *Paratylenchus* (10%), *Trophurus* (7%), *Tylenchorynchus* (27%), *Tylenchus* (40%), *Criconemoides* (4%), *Paratylenchoides* (11%) and *Heterodera* spp (second stage juveniles) were indicated in 211 of the samples collected.

A total of 211 samples were taken from seventeen different regions of Turkey in order to report that 95 samples (45%), 179 samples (84) and 138 samples (65%) from 211 samples were infected with *P. thornei*, *P. neglectus* and *D. dipsaci* in this region (Table 18 and 20).

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Table 18. Nematode per 500 cm³ soil and per 5gm roots of each soil sample of Chickpea collected in Turkey.

Location Name	Samples (Number)	<i>Ditylenchus</i> <i>dipsaci</i> -	<i>Pratylenchus</i> <i>thornei</i>	<i>Pratylenchus</i> <i>neglectus</i> -	<i>Pratylenchus</i> <i>penetrans</i>	Other nematodes
Tekirdağ/Şarköy-yeni köy.	5	240	80	40	6	40 <i>Paratrophurus</i> sp.
Antalya /Elmalı.	5	420	120	80	4	40 <i>Paratrophurus</i> sp.
Burdur/Bucak-kızıl köy.	6	200	200	120	10	60 <i>Tylenchus</i>
Antalya/Dağbelen.	3	60	180	80	-	-
Malatya/ Alpinen .	4	240	20	10	4	40 <i>Tylenchus</i>
Çanakkale/Bayramiç -Soldüz köyü.	7	280	40	15	-	-
Denizli/Serinhisar	4	400	60	20	2	140 <i>Paratylenchus</i> sp. 20 <i>Tylenchus</i>
Tekirdag/Malkara/karal	2	380	20	25	20	40 <i>Paratylenchoides</i> sp.
Bursa Nilüfer/karacadağ köyü	3	60	40	45	8	22 <i>Tylenchus</i>
Muş/merkez-Erencik köyü.	4	80	60	20	-	-
Denizli/Serinhisar yatagan 2	10	300	100	80	16	<i>Tylenchorhynchus</i> sp
Elazığ merkez.	5	220	120	50	4	-
Burdur/Bucak/Karapınar köyü	6	180	140	60	8	60 <i>Paratylenchus</i> sp.
Muğla/Fethiye.	8	420	140	70	11	-
Malatya/Akçadag/kozlucu.	8	140	40	20	12	40 <i>Tylenchus</i>
Balıkesir/Gönen/Buğdaylı.	10	360	80	60	-	40 <i>Paratylenchus</i> sp. 20 <i>Pratylenchoides</i> sp.
Denizli/Serinhisar/Cumhuriyet mah.	8	140	100	30	20	20 <i>Paratylenchus</i> sp. 60 <i>Tylenchus</i>
Çanakkale-Biga kuruaba köyü.	7	20	40	20	-	-
Balıkesir-Bandırma orhaniye köyü.	7	300	100	45	15	60 <i>Tylenchorhynchus</i> sp.

Table 18(Continue Previous Page))

Hatay/Beler-kııcı köyü	11	140	20	5	20	70 <i>Tylenchorhyncs</i> sp. 40 <i>Tylenchus</i>
Balıkesir/Gönen-Buğdaylı köyü.	4	20	40	30	20	20 <i>Criconemoides</i>
Denizli/karahayıt	5	260	80	40	-	-
Muş merkez/yaygın-ziyaret köyü.	5	60	60	20	10	20 <i>Tylenchus</i>
Tunceli/Ovacık-merkez.	3	40	120	100	20	-
Malatya/Doğanşehir.	3	260	200	120	32	-
Çanakkale/Ezine.	2	20	80	20	20	100 <i>Trophurus</i> sp.
Muğla/Fethiye-seki.	5	480	80	204	-	-
Bilecik/Bozüyük/Akpınar.	7	20	80	31	20	60 <i>Paratylenchus</i> sp. 20 <i>Pratylenchoides</i> sp.
Antalya/Korkuteli/Garipa.	8	40	60	36	15	-
Denizli/Tavas1/Çiftli köy.	7	20	20	20	6	20 <i>Paratylenchoides</i> sp.
Elazığ Baskil-karabeyli köyü.	4	40	80	60	20	-
Denizli/Serinhisar/merkez.	4	20	60	30	-	10 <i>Tylenchorhynchus</i> sp.
Burdur/Bucak.	3	20	60	20	20	-
Tekirdag/Malkara/Balı.	8	-	40	45	-	-
Adana.	2	1	2	1	-	10 <i>Xphinema</i> sp.
Ankara/Haymana.	12	2	4	3	-	-
Sanliurfa/Harran university.	6	3	5	4	-	10 <i>Tylenchus</i>
Total	211	95	179	138	106	-
Percentage	-	45%	84%	65%	56%	-

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Table 19. Nematode per 500 cm³ soil and per 5gm of roots in each soil sample of Chickpea.

Location Name	Samples (Number)	<i>Aphelenchus</i> spp	<i>Helicotylenchus</i> spp	<i>Dorylaimid</i> spp	<i>Merlinus</i> spp	Other nematodes
Tekirdağ/Şarköy/yeniköy.	5	40	-	80	140	40 <i>Xphinema</i> sp.
Antalya Elmalı.	5	180	-	40	-	-
Burdur/Bucak(kızıl köy).	6	-	40	100	-	40 <i>Tylenchorhynchus</i> sp.
Antalya/Dağbelen.	3	60	-	60	-	-
Malatya/Alpiner .	4	20	20	100	280	-
Çanakkale/Bayramiç-soldüz köyü).	7	20	-	40	-	20 <i>Tylenchorhynchus</i> sp.
Denizli/Serinhisar.	4	80	100	40	-	<i>Criconemoides</i>
Tekirdag/Malkara/karal.	2	-	-	-	-	-
Bursa Nilüfer/karacadağ köyü.	3	100	40	20	80	20 <i>Trophurus</i>
Muş/merkez/Erencik köyü.	4	180	100	40	-	-
Denizli/Serinhisar yatagan.	10	100	80	20	60	<i>Tylenchus</i> sp.
Elazığ merkez.	5	100	40	160	160	60 <i>Tylenchorhynchus</i> sp.
Buralur/Bucak/Karapinar köyü.	6	480	100	40	20	-
Muğla/Fethiye.	8	40	20	60	-	20 <i>Tylenchus</i> sp.
Malatya/Akçadag/kozluca.	8	20	20	20	30	20 <i>Criconemoides</i>
Balıkesir/Gönen/Buğdaylı.	10	200	100	60	40	-
Denizli/Serinhisar/cumhuri yet mah.	8	110 0	220	100	20	20 <i>Xphinema</i> sp.
Çanakkale-Biga koruaba köyü.	7	40	40	-	180	<i>Tylenchus</i> sp.
Balıkesir-Bandırma orhaniye köyü.	7	40	-	40	-	10 <i>Trophurus</i>
Hatay-Beler/kıcı köyü.	11	40	-	140	40	20 <i>Tylenchorhynchus</i> sp. 40 <i>Tylenchus</i>
Balıkesir/Gönen-Buğdaylı köyü.	4	20	-	-	20	20 <i>Tylenchus</i>

Table 19(Continue Previous Page))

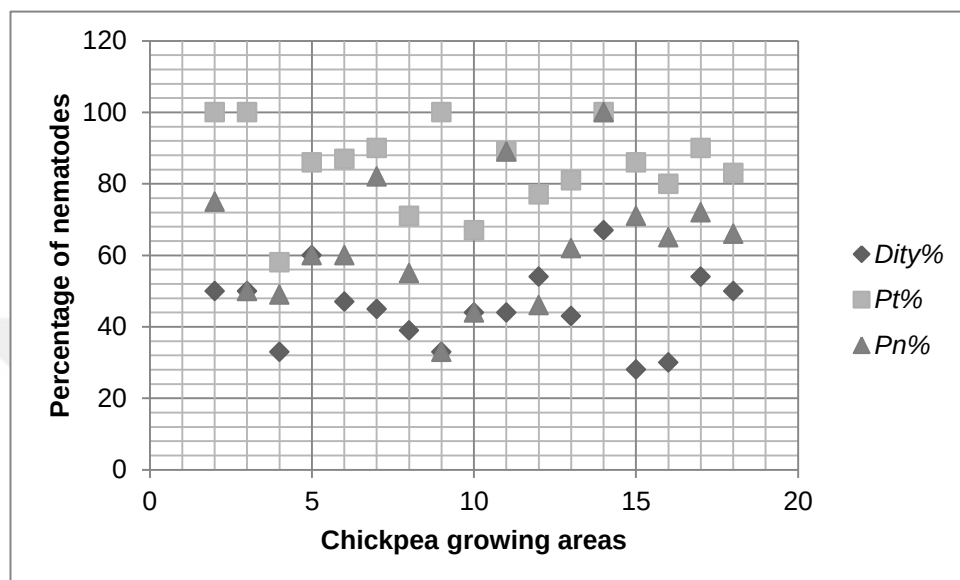
Denizli/karahayıt.	5	20	-	-	20	-
Muş merkez/yaygın-ziyaret köyü.	5	20	40	-	-	20 <i>Tylenchorhynchus</i> sp.
Tunceli/Ovacık/Merkez.	3	20	360	-	-	-
Malatya/Doğanşehir.	3	20	40	-	-	-
Çanakkale/Ezine.	7	-	-	40	80	10 <i>Xphinema</i> sp.
Muğla/Fethiye-Seki.	5	20	-	-	40	10 <i>Trophurus</i>
Bilecik/Bozüyük/Akpınar.	7	200	-	60	80	-
Antalya/Korkuteli/Garipa.	8	20	20	-	20	120 <i>Xphinema</i> sp.
Denizli/Tavas1/Çiftli köy.	7	20	20	20	20	20 <i>Tylenchus</i>
Elazığ Baskil-Karabeyli köyü.	4	20	20	20	-	-
Denizli/Serinhisar/Merkez.	4	40	80	-	140	20 <i>Paratylenchoides</i> sp.
Burdur/Bucak.	3	20	20	20	-	-
Tekirdag/Malkara/Balı.	8	20	20	20	-	100 <i>Xphinema</i> sp.
Adana.	2					
Ankara/Haymana.	12					
Sanliurfa/Harran university.	6					
Total	206	123	95	74	69	-
Percentage		65 %	50 %	39 %	36 %	-

Table 20. The list of distribution by percentage of *Pratylenchus thornei*, *P. neglectus* and *Ditylenchus dipsaci* in different regions of Turkey.

Location name	Total sample	<i>Dity</i> %	<i>Pt</i> %	<i>Pn</i> %
Ankara	4	50	100	75
Adana	2	50	100	50
Antalya	24	33	58	49
Burdur	15	60	86	60
Malatya	15	47	87	60
Canakkale	11	45	90	82
Denizli	38	39	71	55
Bursa	3	33	100	33
Mus	9	44	67	44
Elazig	9	44	89	89
Mugla	13	54	77	46
Balikesir	21	43	81	62
Tunceli	3	67	100	100
Bilecik	7	28	86	71
Tekirdag	20	30	80	65
Hatay	11	54	90	72
Sanliurfa	6	50	83	66

Pt: *P. thornei*Dity: *D. dipsaci*Pn: *P. neglectus*

Figure 4.13. Geographical distribution of the most important plant parasitic nematodes in chickpea growing areas (within seventeen regions of Turkey).



4.2.2. Population dynamics of the root lesion nematodes *P. thornei* (Sher and Allen), *P. neglectus* and *D. dipsaci* on wild cicer species.

In this study, 12 accessions of wild cicer spp. (*C. reticulatum* and *C. echinospermum*) of winter and spring chickpea cultivar have been evaluated the occurrence and population dynamics of the root lesion (*P. thornei*, *P. neglectus*) and *Ditylenchus dipsaci* with four replications. This study was conducted during 2014-2016 at two different regions of Turkey (Sanliurfa and Ankara). A total 12 accessions of wild Cicer sp was evaluated with the population dynamic density increase of root lesions (*P. thornei*, *P. neglectus*) and *D. dipsaci* in *Cicer arietinum* (n=3), *C. echinospermum* (n=4) from 3 collection sites; and *C. reticulatum* (n=5) from 3 collection sites. In order, the average soil temperature in Ankara and Sanliurfa regions study at depth of 20 cm was 7.3°C and 9.6°C from November to February and rose to an average of 16.3°C and 25.9°C from March to July. Also, in order monthly average precipitation in Ankara and Sanliurfa was measured 32.4

mm and 19.7 mm from April to March and 23.3 mm and 72.2 mm from November to February during 2015-17 growing season (TSMS, 2017) (Figure 4.34, 35, 42 and 43).

4.2.2.1. *Pratylenchus thornei*

The resulting variance analysis of the accessions of wild *cicer* spp to *P. thornei* nematode indicated that:

- ✓ There were significant genotypic differences within each species $P < 0.001$ (Table 21 and 22).
- ✓ Wilds *Cicer* spp (*C. reticulatum* and *C. echinospermum*) were more resistant to nematodes than domestic (*Cicer arietinum*) $P < .001$ (Table 21).
- ✓ The most susceptible line was *C. reticulatum* Ak 377. Gokce was the most susceptible *C. arietinum*, equivalent to the mean *C. reticulatum* sensitivity Azkan and AK 32 were the most resistant *Cicer arietinum* and *C. reticulatum*, respectively (Table 30). Both of them were similarly responsive to *P. thornei*, and more susceptible than any *C. echinospermum* genotype (Fig 4.30, 4.31, 4.32 and 4.33).
- ✓ Conclusions of both sites (Ankara and Sanliurfa) were identical and all of the genotypes indicated a same reaction to *P. thornei* nematode. (Figure 4.30, 4.31, 4.32 and 4.33)
- ✓ *C. echinospermum* was more resistant to *P. thornei* than *C. arietinum* and *C. reticulatum* $P < .001$ (Table 21).
- ✓ Within *C. echinospermum* there were no genotypic differences in *P. thornei* sensitivity (Table 30).
- ✓ The time of sowing (Autumn and Spring) tends not to influence nematode responses to *cicer* species and genotypes within species in either Sanliurfa or Ankara. Although, there was no difference observed between the time of

sowing (Spring and Autumn) (Table 21,22 and 23), the population densities of nematode increased more than other planting times, during harvest time in both sites (Figure 4.30, 4.31, 4.32 and 4.33).

- ✓ All stages of nematodes were detected in the roots and soil throughout both growing seasons (Spring and Autumn) in both locations, while the maximum numbers of nematodes were extracted from roots and soil during May and July. The population densities of *P. thornei* was increased from early spring (March) to mid-summer (July) in both regions (Table 21, 22 and Figure 4.34). Moreover, the result of this study indicated that the nematode's population density tends to decrease from the late summer (August) to the end of winter and starts to increase from the early spring (March) to the mid-summer. Generally, the data results indicated that the population density of *P. thornei* nematode was the lowest density in experiment plots during January and February and was the highest density in plots between June and July in both regions (Ankara and Sanliurfa) (Table 21 and 22). It was estimated that the population density of nematodes was increased in the plots in the early spring (March) than in the first sampling results in 100gm of soil in the mid-summer (July) (Figure 4.34 and 4.35). Also, *P. thornei* population density showed a positive correlation with soil temperature (Figure 4.36).

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Table 21. Analysis of variance of *Pratylenchus thornei*

Source of variation	d.f.	s.s	m.s	v.r	F pr
Rep stratum	3	12,429.	4,143.	1.59	
Reps.Tos stratum					
Tos	1	24,131	24,131	9.24	0.056
Residual	3	7,837	2,612	2.56	
Reps.Tos.*Units* stratum					
Obs_no	3	735,404.	245,135.	239.96	<.001
Sp	2	953,191.	476,595.	466.54	<.001
Tos.Obs_no	3	16,293.	5,431.	5.32	0.001
Tos.Sp	2	19,384.	9,692.	9.49	<.001
Obs_no.Sp	6	224,725.	37,454.	36.66	<.001
Sp.AK_Line	9	169,396.	18,822.	18.42	<.001
Tos.Obs_no.Sp	6	11,248.	1,875.	1.84	0.092
Tos.Sp.AK_Line	9	12,725.	1,414.	1.38	0.195
Obs_no.Sp.AK_Line	27	102,515.	3,797.	3.72	<.001
Tos.Obs_no.Sp.AK_Line	27	33,648.	1,246.	1.22	0.214
Residual	279	285,014.	1,022		
Total	380	2,526,127.			

Tos: Time of sowing (Autumn and Spring) Sp: species

Obs: Observation of sampling time Ak line: chickpea genotype

Table 22. Tables of means of *Pratylenchus thornei*

Tos	Spring	Autumn			
	91.4a	107.3a			
LSD	16.60				
Obs_no	Sowing Time (March)	Flowering Time (May)	Podding Time (June)	Harvest Timee (July)	
	49.1a	72.8b	110.5c	165.0d	
LSD	9.08				
Sp	<i>C.arietinum</i>	<i>C.echinespermum</i>	<i>C.reticulatum</i>		
	109.6a	32.1b	147.0c		
LSD	8.12				
Tos	Obs_no	Sowing Time	Flowering Time	Podding Time	Harvest Timee
Spring		46.2a	70.7b	92.1c	156.7d
Autumn		51.9a	75.0b	129.0c	173.2d
LSD	16.50				

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Table 23. Result of least significant differencesb analysis (5% level)

Table	Tos	Obs_no	Sp	Tos Obs_no	
rep.	192	96	unequal	48	
l.s.d.			9.08X		min.rep
d.f.			279		
l.s.d.	16.60	9.08	8.12	16.50	max-min
d.f.	3	279	279	13.96	
l.s.d.			7.03X		max.rep

(No comparisons in categories where l.s.d. marked with an X)

Figure 4.14. Response of 12 accessions of cultivar chickpea against the *Pratylenchus thornei* nematode at the Ankara site (Spring cultivar).

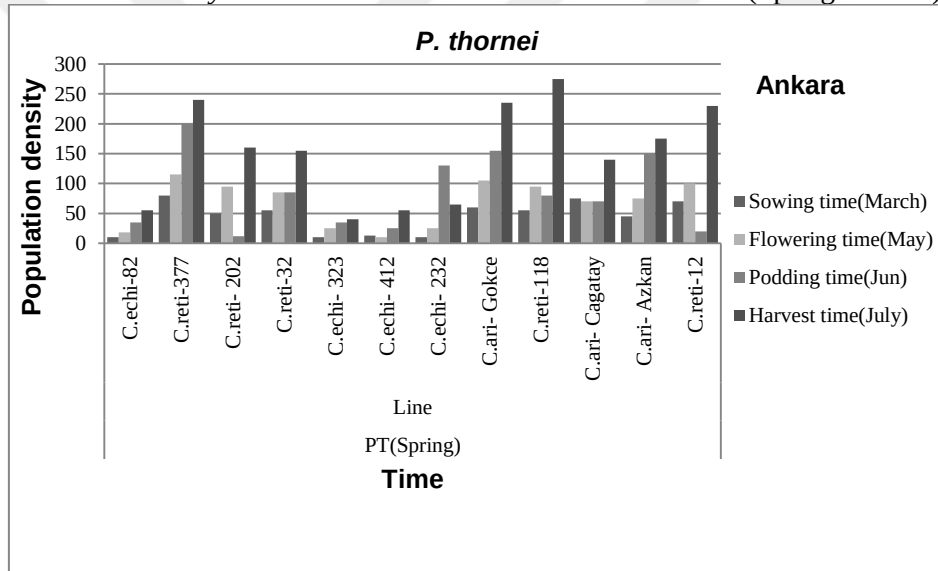


Figure 4.15. Response of 12 accessions of cultivar chickpea against the *Pratylenchus thornei* nematode at the Sanliurfa site (Spring cultivar).

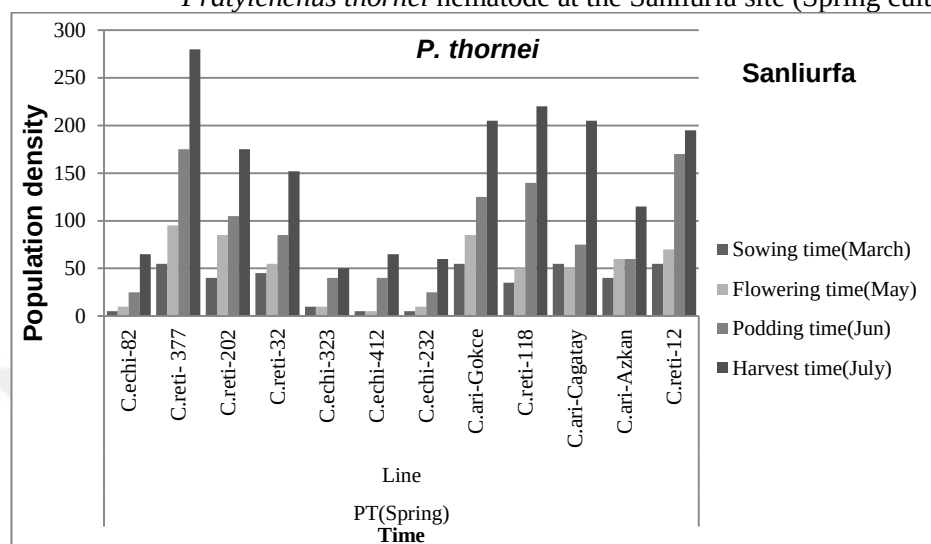


Figure 4.16. Response of 12 accessions of cultivar chickpea against the *Pratylenchus thornei* nematode in the Ankara site (Autumn cultivar).

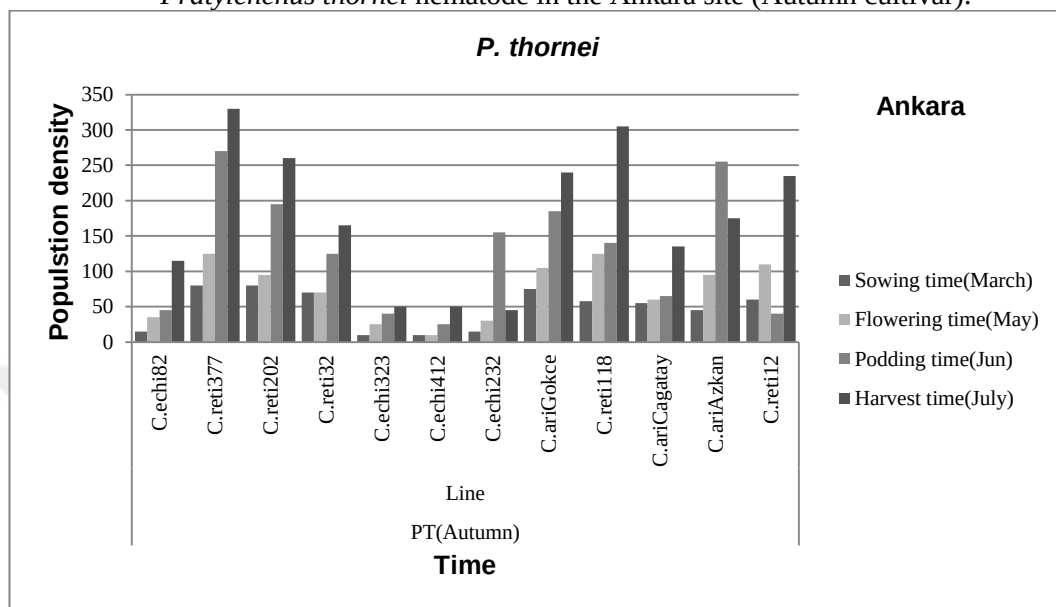


Figure 4.17. Response of 12 accession of cultivar chickpea against the *Pratylenchus thornei* nematode in the Ankara site. (Autumn cultivar).

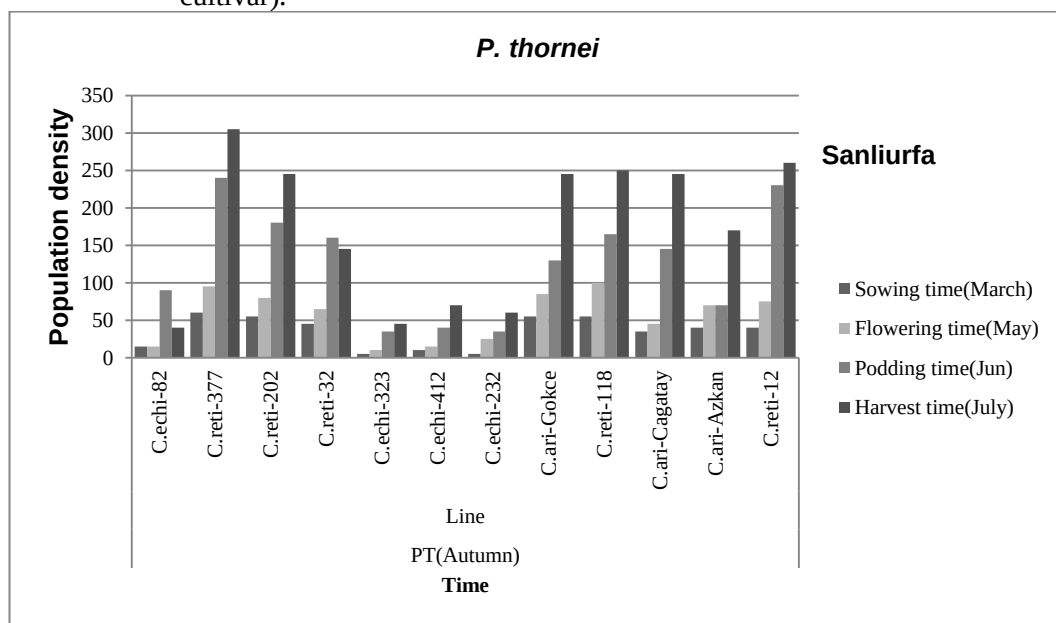


Figure 4.18. The monthly average temperature at 20 cm soil depth, total rainfall and population density of *Pratylenchus thornei* in Ankara during 2015-17.

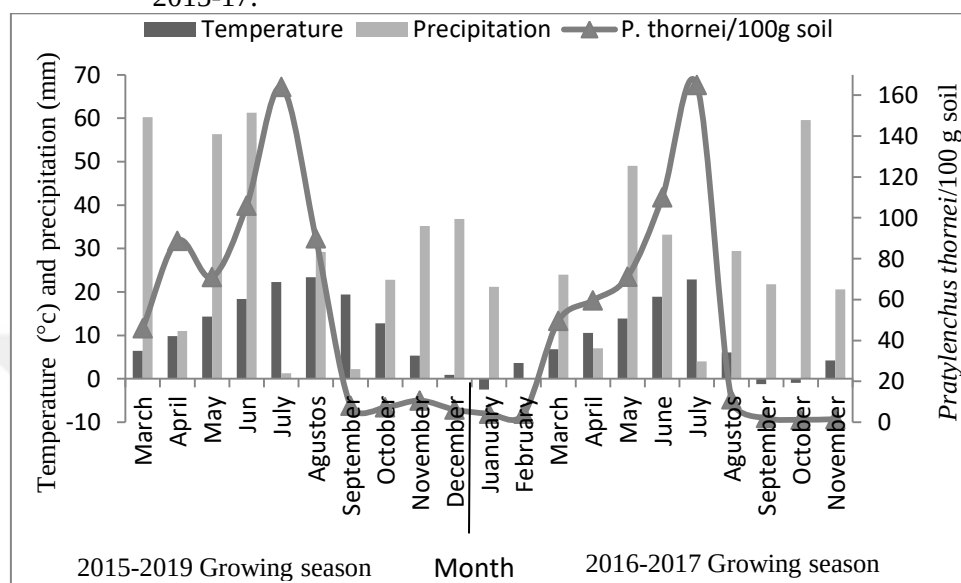


Figure 4.19. The monthly average temperature at 20 cm soil depth, total rainfall and population density of *Pratylenchus thornei* in Sanliurfa during 2015-17.

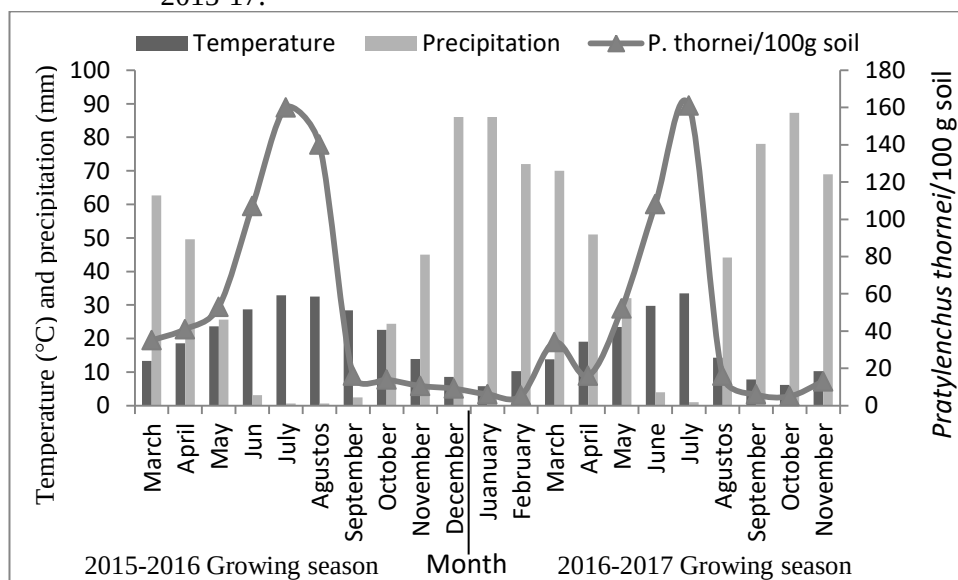
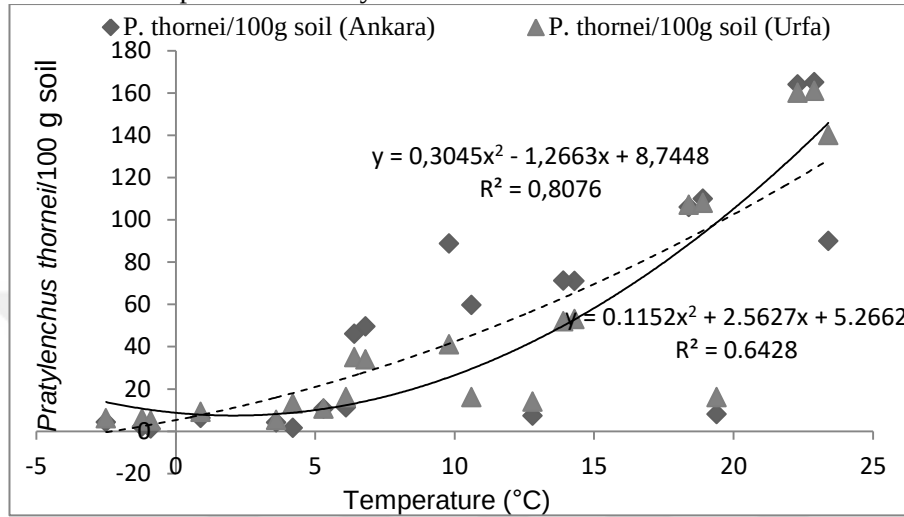


Figure 4.20. Relationship between the monthly mean temperature at 20 cm soil depth and *Pratylenchus thornei* population density during two growing season (November 2015-July 2017) in Ankara and Urfa province Turkey.



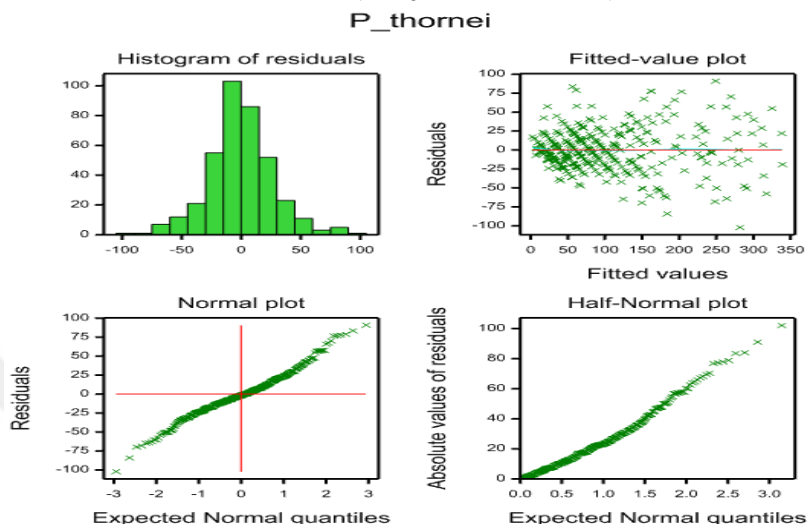
4.2.2.1. (1). The histogram of residual for *Pratylenchus thornei*

The histogram of residuals was used to assess whether residuals had normal or half normal plot distribution in experiment 1 (Figure 4.36). Standardized residuals for all predicted values should be distributed symmetrically. The histogram of residuals must be bell-shaped, like a normal distribution curve and both the normal and half normal plot should be straight lines at 45°. In this instance the residual plot was satisfactory, although, the residual histogram was somewhat skewed to the right, and as a result, there was some deviation from linear in both normal plots. Therefore, there was a reflection of the experimental design.

Figure 4.21. The simplest model for this experiment is a one-way ANOVA:

***** Analysis of variance *****

Variate: Final count of nematodes (*Pratylenchus thornei*)



4.2.2.1. (2). The means of ANOVA for *Pratylenchus thornei*

The histogram of a residual and fan-shaped experiment for the *P. thornei* above, confirmed that there was a significant difference between variants in this experiment. In addition, the residuals were normally distributed as indicated by the skewed histogram, and deviated from linearity in the normal plots. The histogram of residual was much less skewed and the normal plots look much more like straight lines. Also, ANOVA indicates that there was significant treatment of differences in the trial ($P < 0.001$).

4.2.2.2. *Pratylenchus neglectus*

The resulting analysis of variance in accessions of wild *Cicer* spp. to *P. neglectus* nematode indicated that:

- ✓ Big species differences in *P. neglectus* sensitivity. *P. neglectus* numbers increase more rapidly over time in *C. echinospermum* compared to *C. reticulatum* and *C. arietinum* $P < .001$ (Table 24 and 25).

- ✓ Wild *cicer* spp (*C. reticulatum* and *C. echinospermum* were more resistant to nematodes than domestic *C. arietinum* ($P < 0.001$) (Table 24).
- ✓ However, unlike in Sanliurfa, there was evidence of a 4-way interaction: the rate of *P. neglectus* increases and varies across accessions within species over planting time (Table 24 and Figure 4.42).
- ✓ There were 2 *C. echinospermum* genotypes (Ak 82, 323) that were particularly susceptible to *P. neglectus* in the autumn planting with nematode numbers rising sharply at the end of the season (Table 30 and Figure 4.39 and 4.40). In contrast, autumn-planted Ak 412, 232 were as resistant as susceptible *C. reticulatum* (Table 30 and Figure 4.38).
- ✓ *Cicer arietinum* was the most resistant at both times of sowing (Autumn and Spring) (Figure 4.38, 4.39, 4.40, 4.41).
- ✓ *Cicer reticulatum* had a wider range of responses to *P. neglectus* than chickpea ($P < 0.001$) (Table 24). Ak 377, 202 and 12 were susceptible to *P. neglectus* in both sowing times (Autumn and Spring). Moreover, Ak 32 and 118 to a lesser extent were consistently resistant, as was the case in Sanliurfa (Table 30 and Figure 4.39).
- ✓ The time of sowing (Autumn and Spring) tends to not influence nematode responses to *cicer* species ($P < 0.363$) and the genotypes within species in either Sanliurfa or Ankara ($P < 0.034$) (Figure 4.38, 4.39, 4.40 and 4.41). The exception was *P. neglectus* in Ankara, where there was susceptible/resistance divergence in Autumn-sown *C. echinospermum*. Also results indicated there were no genotypic differences to nematode sensitivity in *C. echinospermum*, except for autumn-sown *P. neglectus* response in Ankara only (Figure 4.39).
- ✓ The population densities of *P. neglectus* was increased such as *P. thornei* from early spring (March) to mid-summer (July) in both regions ($P < 0.001$) (Table 24 and 25 and Figure 4.42 and 4.43). Moreover, the result of this study indicated that the nematode's population density tends to decrease

from the late summer (August) to the end of winter and starts to increase from the early spring (March) to the mid-summer. Normally, the data results indicated that the population density of *P. neglectus* nematode was the lowest density in experiment plots during January and February and was the highest density in plots between June and July in both study regions (Ankara and Sanliurfa) (Table 24, 25, 26 and Figure 4.41 and 4.42). It was estimated that the population densities of nematode increased in the plots in the early spring (March) than in the first sampling results in 100gm of soil in the mid-summer (July) (Figure 4.42 and 4.43). Also, *P. neglectus* population density showed a positive correlation with soil temperature (Figure 4.44).

Table 24. Analysis of variance of *Pratylenchus neglectus*

Source of variation	d.f.	s.s	m.s	v.r	F pr
Rep stratum	3	1,209.9	403.3	0.66	
Reps.Tos stratum					
Tos	1	446.3	446.3	0.73	0.457
Residual	3	1845.7	615.2	0.98	
Reps.Tos.*Units* stratum					
Obs_no	3	96,902.4	32,300.8	51.21	<.001
Sp	2	20,911.3	10,455.7	16.58	<.001
Tos.Obs_no	3	3,446.5	1,148.8	1.82	0.143
Tos.Sp	2	1,283.0	641.5	1.02	0.363
Obs_no.Sp	6	30,447.0	5,074.5	8.05	<.001
Sp.AK_Line	9	22,899.7	2,544.4	4.03	<.001
Tos.Obs_no.Sp	6	665.9	111.0	0.18	0.983
Tos.Sp.AK_Line	9	11,658.0	1,295.3	2.05	0.034
Obs_no.Sp.AK_Line	27	15,797.7	585.1	0.93	0.572
Tos.Obs_no.Sp.AK_Line	27	25,364.7	939.4	1.49	0.060
Residual	282	177,871.4	630.7		
Total	383	410,749.7			

Tos: Time of sowing (Autumn and Spring)

Sp: species

Obs: Observation sampling time

Ak line: Cultivars chickpea

Table 25. Table of means of *Pratylenchus neglectus*

Tos	Spring	Autumn			
	49.8a	52.0a			
LSD	8.06				
Obs_no	Sowing Time (March)	Flowering Time (May)	Podding Time (June)	Harvest Timee (July)	
	35.8a	40.6a	50.4b	76.9c	
LSD	7.14				
Sp	<i>C.arietinum</i>	<i>C.echinospermum</i>	<i>C.reticulatum</i>		
	44.1a	61.2b	46.8c		
LSD	6.38				
Tos	Obs_no	Sowing Time (March)	Flowering Time (May)	Podding Time (June)	Harvest Time (July)
Spring		36.2a	41.0a	44.2a	77.9b
Autumn		35.3a	40.2a	56.7b	75.8c
LSD	10.29				

Table 26. Result of least significant differences analysis (5% level).

	Tos	Obs_no	Sp	Tos	
rep.	192	96	unequal	Obs_no	
l.s.d.			7.14X	48	min.rep
d.f.			282		
l.s.d.	8.06	7.14	6.38	10.29	max-min
d.f.	3	282	282	45.28	
l.s.d.			5.53X		max.rep
d.f.		282			

(No comparisons in categories where l.s.d. marked with an X)

Figure 4.22. Response of 12 accessions of native chickpea against the *Pratylenchus neglectus* at Ankara (Spring cultivar).

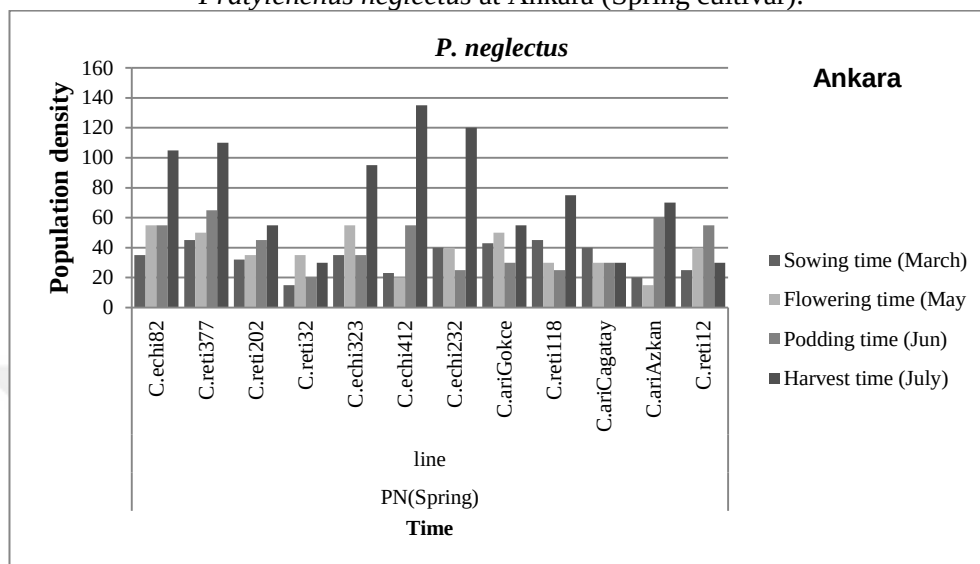


Figure 4.23. Response of 12 accessions of chickpea cultivar against the *Pratylenchus neglectus* at Sanliurfa (Spring cultivar).

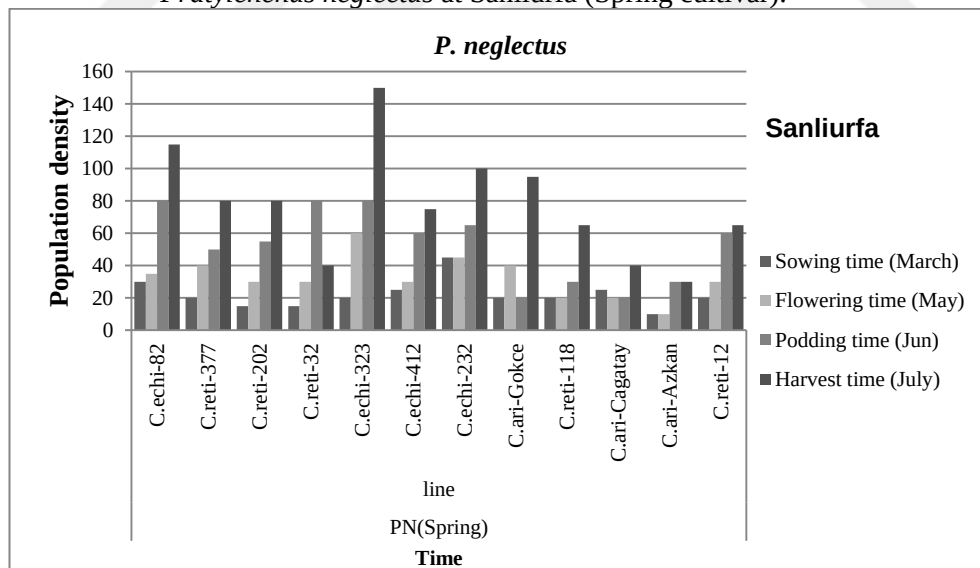


Figure 4.24. Response of 12 accessions of cultivar chickpea against the *Pratylenchus neglectus* at the Ankara (Autumn cultivar).

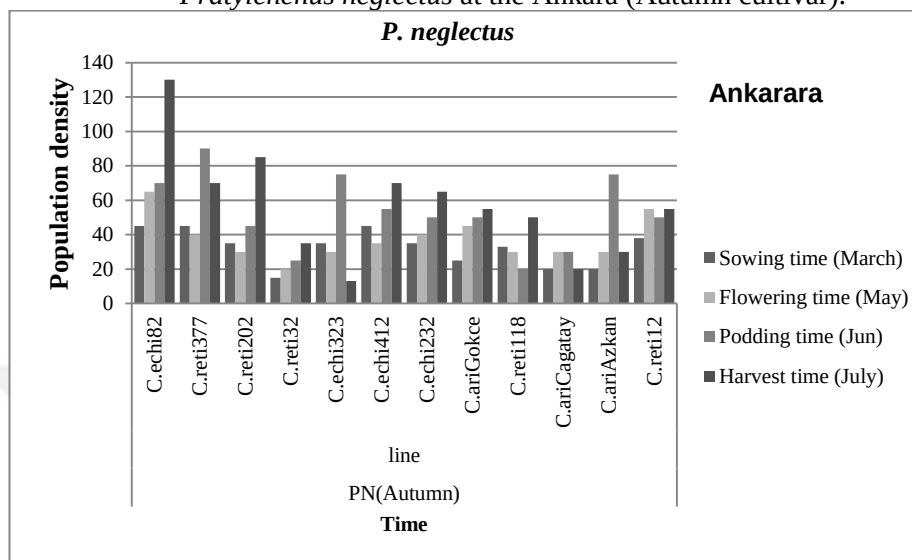


Figure 4.25. Response of 12 accessions of cultivar chickpea against the *Pratylenchus neglectus* at the Sanliurfa (Autumn cultivar).

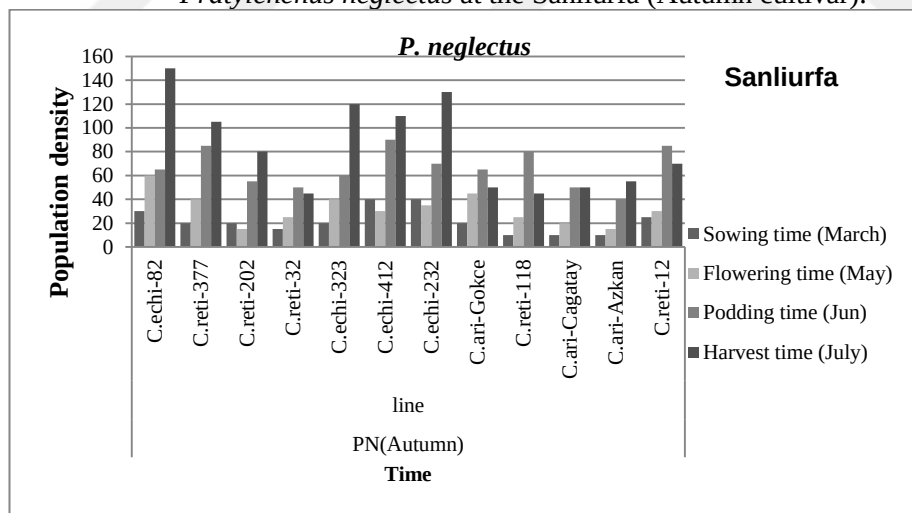


Figure 4.26. The monthly average temperature at 20 cm soil depth, total rainfall and population density of *Pratylenchus neglectus* in Ankara during 2015-17.

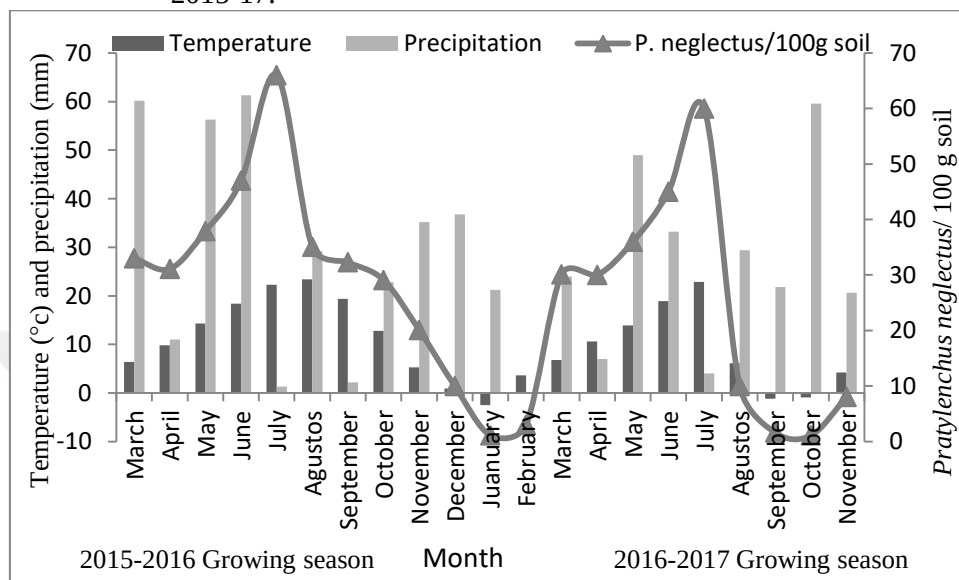


Figure 4.27. The monthly average temperature at 20 cm soil depth, total rainfall and population density of *Pratylenchus neglectus* in Sanliurfa during 2015-17.

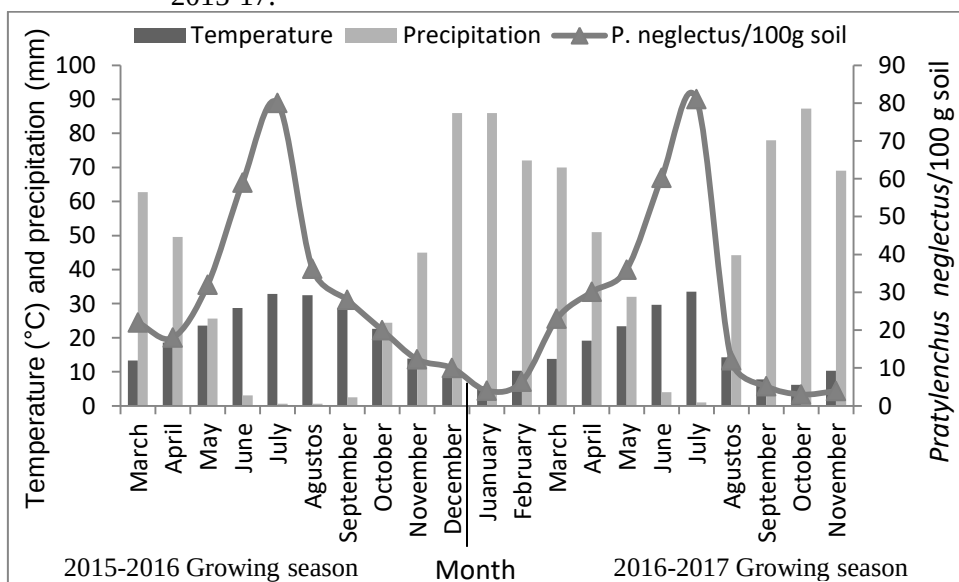
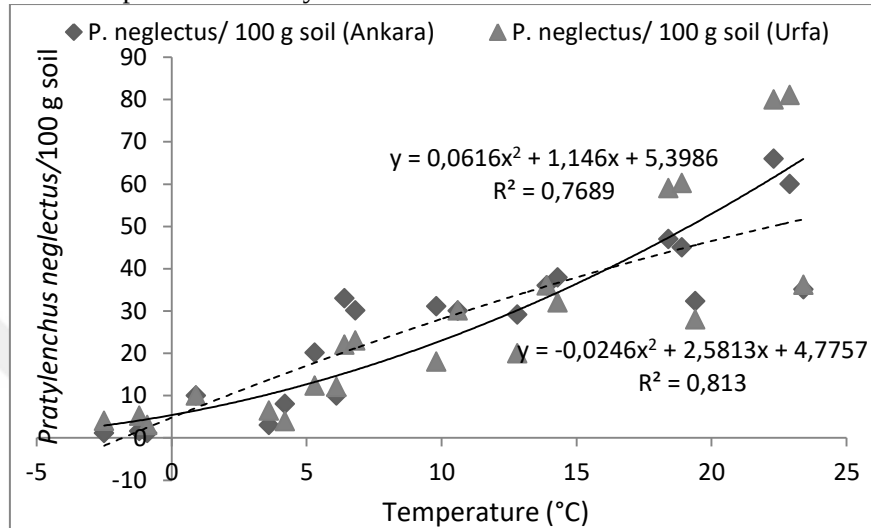


Figure 4.28. Relationship between the monthly mean temperature at 20 cm soil depth and *Pratylenchus neglectus* population density during two growing season (November 2015-July 2017) in Ankara and Urfa province Turkey.



4.2.2.2. (1). Analysis (one-way ANOVA) of *Pratylenchus neglectus*

The histogram of residuals was used to assess whether residuals had normal or half normal plot distribution in experiment 1 (Figure 4.45). Standardized residuals for all predicted values should be distributed symmetrically. The histogram of residuals must be bell-shaped, like a normal distribution curve and both the normal and half normal plot should be straight lines at 45°. In this instance the residual plot was satisfactory, although, the residual histogram was somewhat skewed to the right, and as a result, there was some deviation from linear in both normal plots. Because of that, there was a reflection of the experimental design.

4.2.2.2. (2). The means of ANOVA for *Pratylenchus neglectus*.

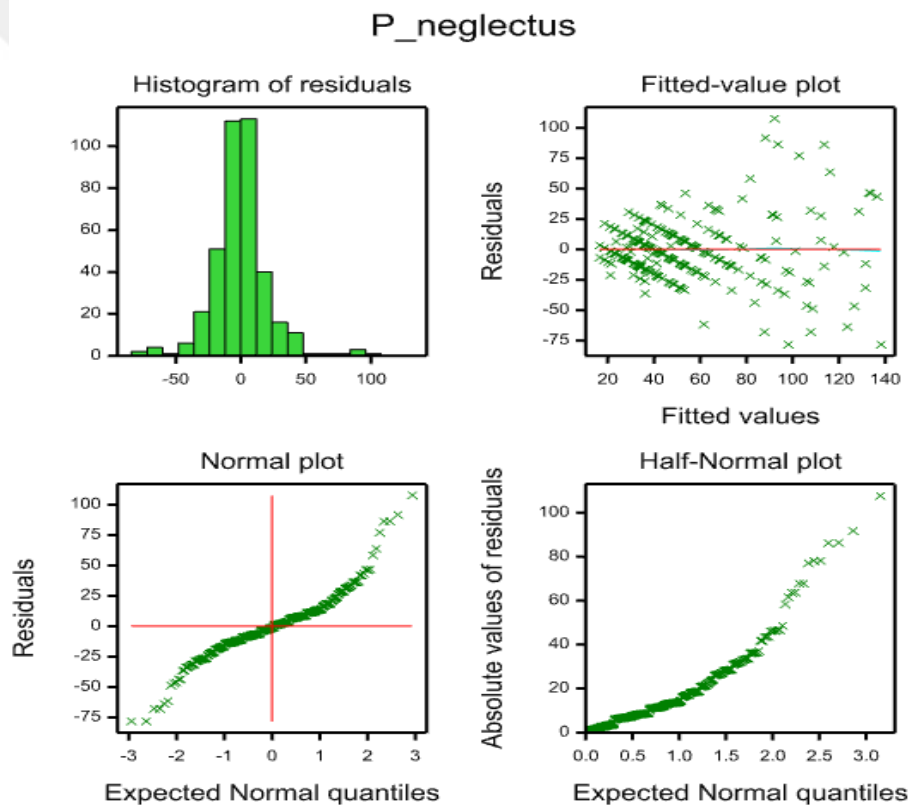
The histogram of a residual and fan-shaped experiment for *P. neglectus* confirmed that there was the significant difference between variance in this

experiment. In addition, the residuals were normally distributed as indicated by the skewed histogram, and deviation from linearity in the normal plots. The histogram of residual was much less skewed and the normal plots look much more like straight lines. Also, ANOVA indicates that there was significant treatment of differences in the trial ($P < 0.001$).

Figure 4.29. The simplest model for this experiment is a one-way ANOVA

***** Analysis of variance *****

Variate: Final count of nematodes (*Pratylenchus neglectus*).



4.2.2.3. *Ditylenchus dipsaci*

The resulting variance analysis of accession of wild *cicer* spp. to *D. dipsaci* is nematode indicated that:

- ✓ There were a significant species differences in *D. dipsaci* sensitivity $P < 0.001$ (Table 27, 28 and 29), also the population densities of *D. dipsaci* nematode numbers were slower to increase over time in *C. echinospermum* compared to *C. reticulatum* and *C. arietinum* (Table 28 and 29).
- ✓ The time of sowing (Autumn and Spring) tends to not influence nematode responses to *cicer* species $P < 0.010$ and the genotypes within species in either Sanliurfa or Ankara $P < 0.235$ (Table 27)
- ✓ There were smaller, but significant genotypic differences within *cicer reticulatum* and *C. arietinum*. *C. reticulatum* (Ak 118, 377) and *C. arietinum* Gocke were very susceptible (Table 27).
- ✓ *Cicer reticulatum* Ak 32, 202 and *C. arietinum* Azkan and Catagay were more resistant, but were not as much as any *C. echinospermum* genotype (Table 30).
- ✓ There was not any indicated or observed significant difference genotypic in *C. echinospermum* (Table 30).
- ✓ The wild *cicer* spp (*C. reticulatum* and *C. echinospermum*) were more resistant to nematodes than domestic species (*C. arietinum*) (Table 28 and 29).
- ✓ There was a significant difference between observation sampling time $P < 0.001$ (March, May, June and July) (Table 27). A similar result was indicated in the *D. dipsaci* nematode. The population densities of *P. neglectus* were increased such as root lesion nematodes (*P. thornei* and *P. neglectus*) from early spring (March) to mid-summer (July) in both regions (Table 28, 29 and Figure 4.49). Generally, the analysis result's data showed that the population density of *D. dipsaci* nematode had the lowest

density in experiment plots during January and February and had the highest density in plots between June and July in both regions (Ankara and Sanliurfa) (Table 28 and Figure 4.51 and 4.52).



Table 27: Analysis of variance of *Ditylenchus dipsaci*

Source of variation	d.f.	s.s	m.s	v.r	F pr
Rep stratum	3	41013.	13671.	21.47	
Reps.Tos stratum					
Tos	1	3234.	3234.	5.08	0.110
Residual	3	1910.	637.	0.58	
Reps.Tos.*Units* stratum					
Obs_no	3	572,944.	190,981.	172.65	<.001
Sp	2	335,402.	167,701.	151.61	<.001
Tos.Obs_no	3	11,390.	3,797.	3.43	0.017
Tos.Sp	2	10,451.	5,226.	4.72	0.010
Obs_no.Sp	6	134,194.	22,366.	20.22	<.001
Sp.AK_Line	9	50,529.	5614.	5.08	<.001
Tos.Obs_no.Sp	6	8,960.	1493.	1.35	0.235
Tos.Sp.AK_Line	9	5,020.	558.	0.50	0.871
Obs_no.Sp.AK_Line	27	46,524.	1,723.	1.56	0.042
Tos.Obs_no.Sp.AK_Line	27	19,817.	734.	0.66	0.899
Residual	278	307,514.	1106		
Total	379	1,544,022.			

Tos: Time of sowing (Autumn and Spring) Sp: species

Obs: Observation sampling time Ak line: Cultivars chickpea

4. RESULTS

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Table 28. Table of means of *Ditylenchus dipsaci*.

Tos	Spring	Autumn			
	86.1a	91.9a			
LSD	8.20				
Obs_no	Sowing Time (March)	Flowering Time (May)	Podding Time (June)	Harvest Timee (July)	
	46.5a	61.7b	101.9c	146.0d	
LSD	9.45				
Sp	<i>C.arietinum</i>	<i>C.echinospermum</i>	<i>C.reticulat um</i>		
	96.0a	48.9b	116.9c		
LSD	8.45				
Tos	Obs_no	Sowing Time	Flowering Time	Podding Time	Harvest Time
Spring		45.8a	62.3b	89.6c	146.7d
Autumn		47.1a	61.0b	114.2c	145.3d
LSD	12.75				

Table 29. Result of least significant differences analysis (5% level)

Table	Tos	Obs_no	Sp	Tos Obs_no	
rep.	192	96	unequal	48	
l.s.d.			9.45X		min.rep
d.f.			278		
l.s.d.	8.20	9.45	8.45	12.75	max-min
d.f.	3	278	278	89.53	
l.s.d.			7.32X		max.rep
d.f.		278			

Figure 4.30. Response of 12 accessions of cultivar chickpea against the *Ditylenchus dipsaci* in he Ankara (Spring cultivar).

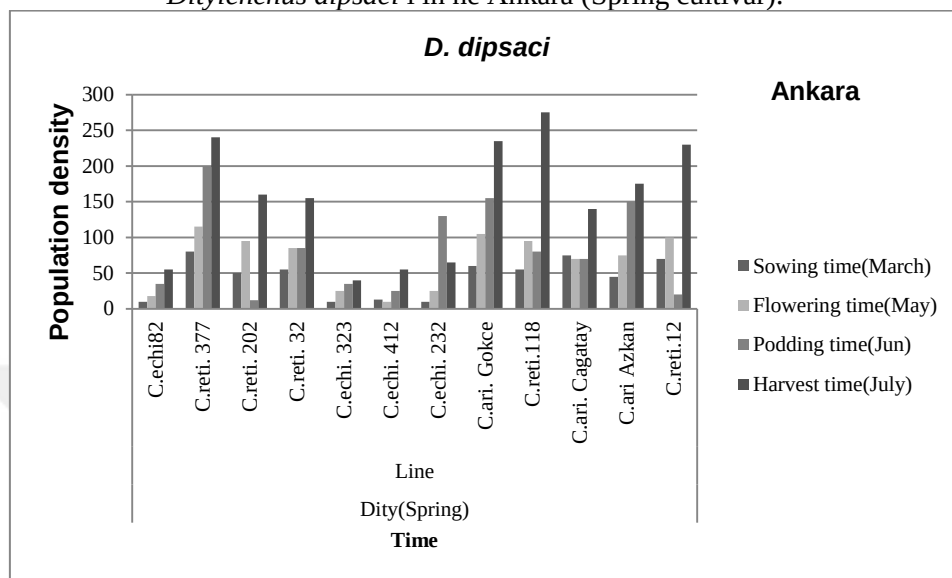


Figure 4.31. Response of 12 accessions of cultivar chickpea against the *Ditylenchus dipsaci* in the Sanliurfa (Spring cultivar)

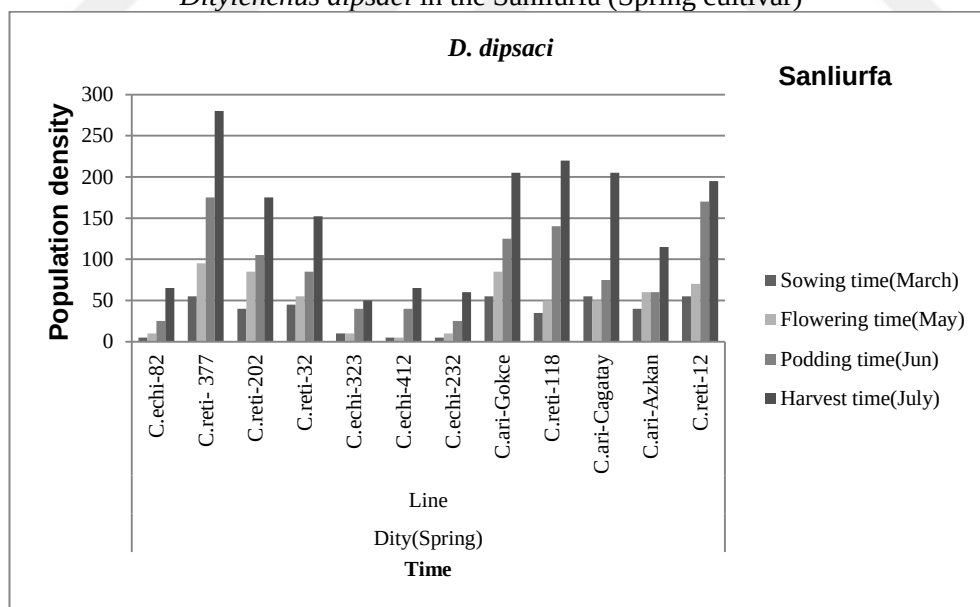


Figure 4.32. Response of 12 accessions of cultivar chickpea against the *Ditylenchus dipsaci* nematode in the Ankara (Autumn cultivar)

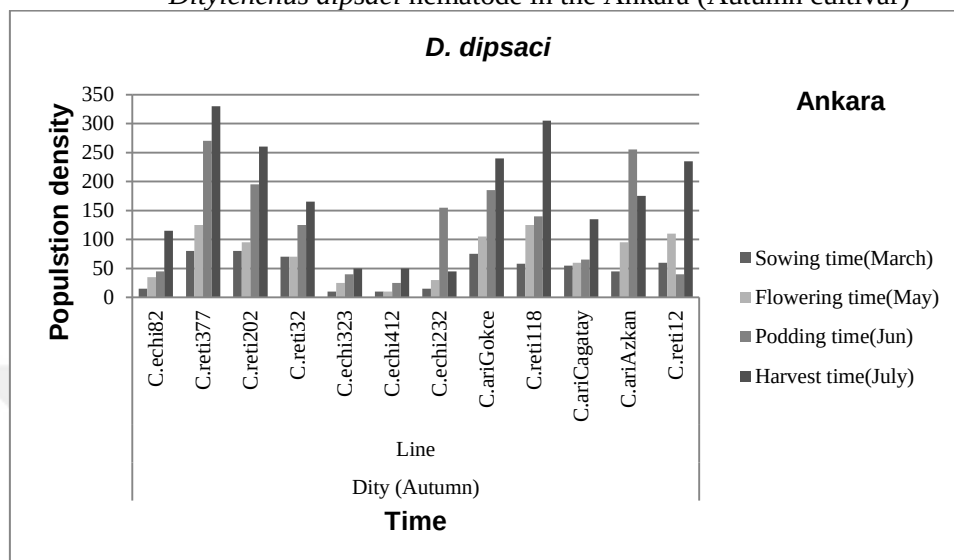
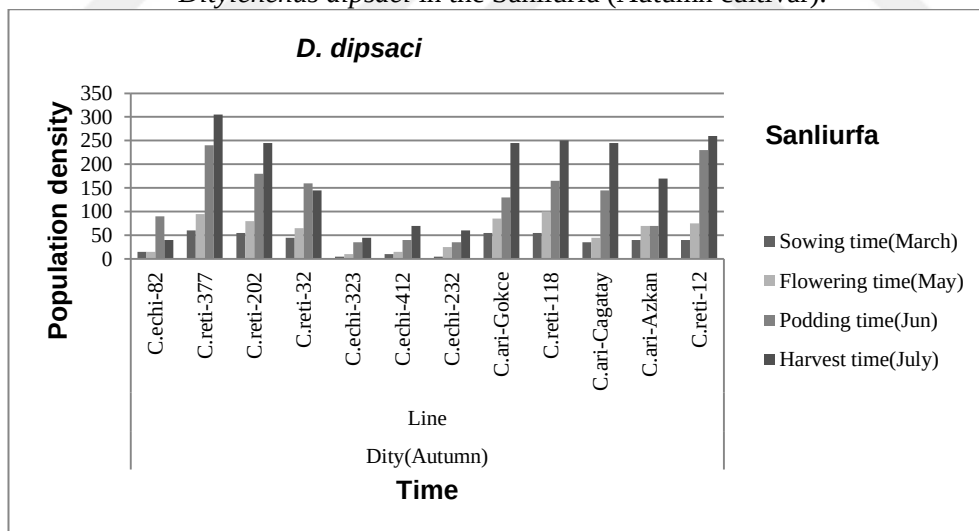


Figure 4.33. Response of 12 accession of cultivar chickpea against the *Ditylenchus dipsaci* in the Sanliurfa (Autumn cultivar).



4.2.2.3. (1). Analysis (one-way ANOVA) of *Ditylenchus dipsaci*

The histogram of residuals was used to assess whether residuals had normal or half normal plot distribution in the experiment (Figure 4.48). Standardized residuals for all predicted values should be distributed symmetrically. The histogram of residuals must be bell-shaped, like a normal distribution curve and both the normal and half normal plot should be straight lines at 45°.

4.2.2.3. (2). The means of ANOVA for *Ditylenchus dipsaci* nematode

The histogram of residual and fan-shaped experiment for *D. dipsaci* confirmed that there was significant difference between variance in this experiment. In addition, the residuals were normally distributed as indicated by the skewed histogram, and deviation from linearity in the normal plots (Figure 4.50). The histogram of residual was much less skewed and the normal plots look much more like straight lines. Also, ANOVA indicates that there was significant treatment of differences in the trial ($P < 0.001$).

Figure 4.34. The simplest model for this experiment is a one-way ANOVA:

***** Analysis of variance *****

Variate: Final count of nematodes (*Ditylenchus dipsaci*)

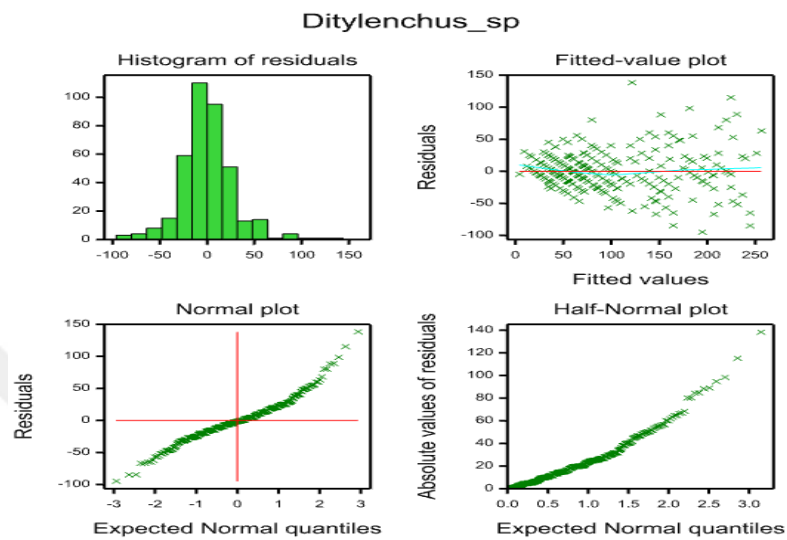


Figure 4.35. The monthly average temperature at 20 cm soil depth, total rainfall and population density of *Ditylenchus dipsaci* in Ankara during 2015-17.

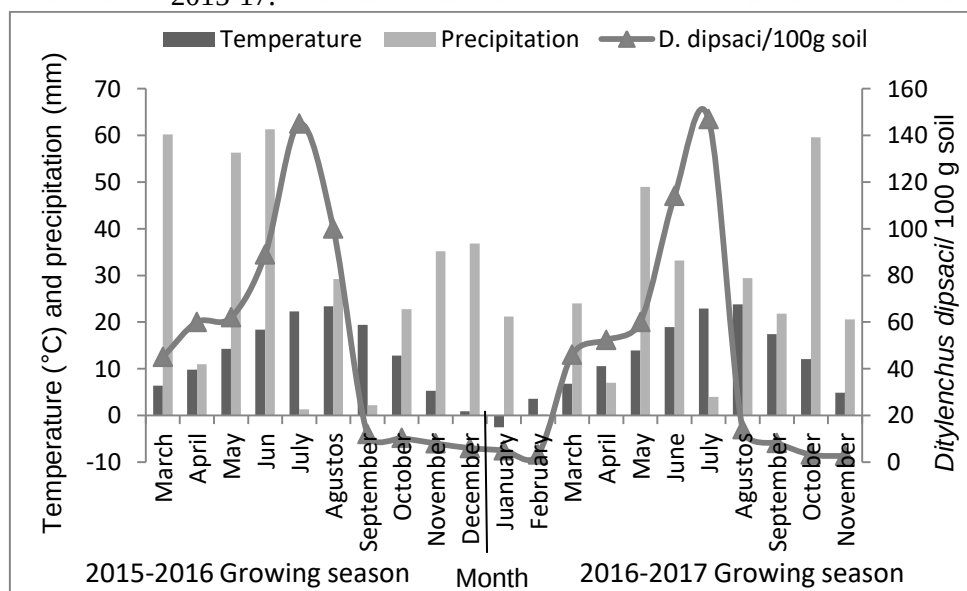
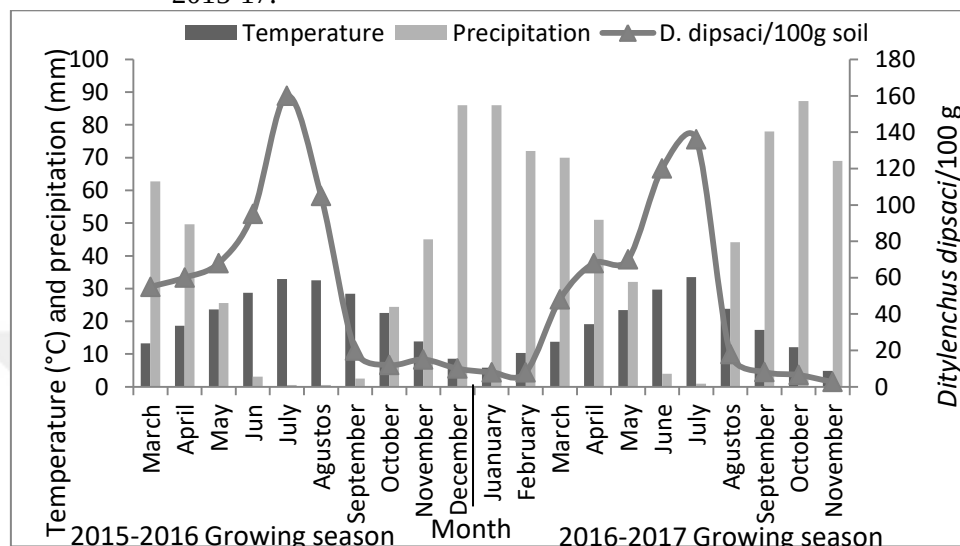


Figure 4.36. The monthly average temperature at 20 cm soil depth, total rainfall and population density of *Ditylenchus dipsaci* in Sanliurfa during 2015-17.



4.2.2.4. Population dynamics of nematodes

The result of population dynamics study indicated that there were species and genotypic differences in nematode sensitivity in the genus *cicer* $P < 0.001$ (Table 27), such that there must be differences in nematode population growth rates and the final number that may interact with location and growing date (Table 28). Also, there were large species differences in nematode sensitivity $P < 0.001$ (Table 27). *C. echinospermum* was more resistant to *P. thornei* and *D. dipsaci*, but more susceptible to *P. neglectus* than *C. arietinum* and *C. reticulata* (Table 28). In addition, the time of sowing (Autumn and Spring) tends not to influence nematode responses to *cicer* species $P < 0.010$ and genotypes within species in either Sanliurfa or Ankara $P < 0.235$ (Table 27). The exception was *P. neglectus* in Ankara, where there was susceptible/resistance divergence in Autumn-grown *C. echinospermum*. Also, the results indicated that there were no genotypic differences to nematode sensitivity in *C. echinospermum*, except for autumn-grown *P. neglectus* response in Ankara only (Figure 4.39). Also, the population density of RLN and *D. dipsaci*

were low at sowing time from March during the cold season period and beginning of the growing season to April, then was increasing gradually to end of growing season (flowering, potting and harvest time) when the air temperature starts to increase and so does the soil temperature, this showed a positive correlation with population densities of RLN in this study (Figure 4.36 and 4.44). Similarly, Elekcioglu and Gozel (1997) reported that the numbers of *P. thornei* in the soil were low until March and then increased during the growing season in Adana Province, Turkey. work (Imren et al., 2017 and Sahin et al., 2008) have shown a correlation between soil temperature and *P. thornei* populations in that nematode populations were low from November to March/April and increased to June/July in Ankara Province, Turkey. The initial sampling was in March 2015 - 2017 during the cold season through to the end of growing season in July in the Ankara province.

Table 30. Field responses to *P. thornei*, *P. neglectus* and *D. dipsaci* populations in Ankara^A and Sanliurfa^U

Nematode	Resistant genotype	Susceptible genotype
<i>C. arietinum</i>		
<i>P. thornei</i>	Azkan ^{A,U}	Gokce ^{A,U}
<i>P. neglectus</i>	No sig diff	No sig diff
<i>D. dipsaci</i>	Azkan ^{A,U} and Catagay ^{A,U}	Gocke ^{A,U}
<i>C. reticulatum</i>		
<i>P. thornei</i>	AK 32 ^{A,U}	AK 377 ^{A,U}
<i>P. neglectus</i>	AK 32 ^{A,U} , 118 ^{A,U}	AK 377 ^{A,U}
<i>D. dipsaci</i>	AK 32 ^{A,U}	AK 118 ^{A,U} , 377 ^{A,U}
<i>C. echinospermum</i> (Ankara only)		
<i>P. neglectus</i> (Autumn only)	AK 412, 232	AK 82, 323

5. DISCUSSION

5.1. Laboratory experiment

5.1.1. Screening and optimization of chickpea genotypes against the root lesion nematode (*P. neglectus*, *P. thornei*) and *D. dipsaci*.

This trial was the first published to study for the infection of Chickpea accessions by root lesion nematodes. Both species of lesion nematodes *P. thornei* and *P. neglectus* have been selected as inoculum as the most important *Pratylenchus* species. Standardization of factors was important for the development of a screening methodology that was stable and definite in distinguishing future various levels of resistance.

Research (Delaville et al., 1996) has shown that factors like soil texture, soil temperature, application technique and harvest time can affect the sensitivity and reproducibility of the screening method. These factors need to be carefully optimized. To protect from such effects, this experiment can be well applied under further standardized conditions in the growth chamber where temperature and light can be controlled. The aim of this study is to choose accessions as providers of resistance for chickpea breeding. The use of nematode numbers to control the level of nematode population density in resistance experiments is extremely important.

As a result, this study indicated that inoculation of each plant by 225 of RLN (*P. thornei* and *P. neglectus*) and 300 inoculum density of *D. dipsaci*, use of sandy soil type (70: 29: 1 sand, field soil and organic matter), 16 weeks harvest time (Figure 4.18), and small tube sizes of 15×100 were the best selection for screening study. The optimization method has been tested and takes an initial condition identified through screening to create an efficient optimization method for screening of Chickpea varieties.

5.1.2. Evaluation of wild *cicer* species (*C. reticulatum* and *C. echinospermum*) against to *P. thornei*, *P. neglectus* and *D. dipsaci* for resistance.

The analyses of variance indicated significant difference between *Cicer* species within nematode species and collection sites where $P < 0.001$ (Table 11 and 14). The accessed collection site within the *Cicer* species indicated that there was significant difference between *Cicer* species within collection sites. There were specific and genotypic differences in nematode sensitivity in the genus *Cicer*, such that there will be differences in nematode population growth rates and final numbers. The results of this study observed that there were large species differences in nematode sensitivity. An inoculum initial density of 225 nematodes per plant (genotype) showed that reproduction rate or multiplication rate between chickpea species within nematode species were different ($P < 0.001$). *C. echinospermum* was more resistant than *C. reticulatum* to *D. dipsaci*, but more susceptible to *P. neglectus* than *C. reticulatum*. *C. reticulatum* tends to be more resistant to *P. neglectus* but more susceptible to *D. dipsaci* than *C. echinospermum* (Table 11 and 14). The chickpea primary gene pool *C. reticulatum* had a distribution of accessions tending towards greater resistance to *P. neglectus* than had *C. echinospermum*. However, there was a considerable genotypic variation for these responses in *C. reticulatum*, such that some genotypes may be more resistant or susceptible than others (Table 17).

5.2. Field study

5.2.1. Plant-parasitic nematodes associated with chickpea in Turkey

Root lesion nematodes *Pratylenchus* spp. are observed worldwide and infest a wide range of plant species. *Pratylenchus* species was first reported by Filipjev (1936) with several revisions since then; e.g. Luc 1987; Frederick and Tarjan 1989. This study indicated that several nematodes were commonly associated with Chickpea in Turkey, among them root lesion nematodes were available in all sampled fields. The literature Di Vito et al. (1992) have shown that

among lesion nematodes, the *P. thornei* can be responsible for the loss of up to 50% of chickpea yields in fields that are infested by this nematode in Syria.

Root lesion nematodes are widely spread in all of chickpea growing areas in Turkey. Different species of root lesion nematodes were detected in various parts of the Chickpea field among them the *P. thornei* and *P. neglectus* play an important role in the economy at the national level and had been reported from many countries infesting cereal and legumes. In these species, the nematodes reduce the resistance plants and damage by feeder roots (Orion et al., 1982). In addition, root lesion nematodes are one of the greatest threats for chickpea production after root-knot nematodes (Barker & Noe, 1987; Jatala and Bridge, 1990). The root lesion nematode, *P. thornei* and *P. neglectus* are a big problem in the Australian grain region too (Riley & Wouts 2001; Riely & Kelly 2002; Hollaway et al. 2008; Thompson et al., 2010).

5.2.2. Population dynamics of the root lesion nematodes *P. thornei* (Sher and Allen), *P. neglectus* and *D. dipsaci* on wild cicer species.

- ✓ There were big species differences in nematode sensitivity. *C. echinospermum* was more resistant to *P. thornei* and *D. dipsaci*, but more susceptible to *P. neglectus* than *C. arietinum* and *C. reticulatum* (Table 22 and 28).
- ✓ Data analyses from Sanliurfa and Ankara gave remarkably consistent results (Table 21, 24 and 27).
- ✓ The time of sowing (Autumn and Spring) tends not to influence nematode responses to *cicer* species and genotypes within species in either Sanliurfa or Ankara (Table 21, 24 and 27). The exception was *P. neglectus* in Ankara, where there was susceptible/resistance divergence in Autumn-grown *C. echinospermum* $P < 0.983$ (Table 24).

- ✓ There were no genotypic differences to nematode sensitivity in *C. echinospermum*, except for Autumn-sown *P. neglectus* response in Ankara only $p < 0.0572$ (Table 24).
- ✓ Between both *cicer* species, *C. arietinum* tends to be more resistant to *P. neglectus*, but observed a sensitivity to *P. thornei* than *C. reticulatum*, with similar responses to *D. dipsaci*. However, there was a considerable genotypic variation for these responses in *C. reticulatum*, such that some genotypes may be more resistant or susceptible than others (Table 22, 25 and 28).
- ✓ There were consistently multiple resistant and susceptible genotypes in both species across both locations (Table 21, 24 and 27). All of the genotypes that were susceptible and resistant to these nematodes in both chickpea cultivars (*C. reticulatum* and *C. echinospermum*) have been listed (Table 30).
- ✓ All stages of nematodes were detected in roots and soil throughout both growing seasons, while the maximum number of nematodes were extracted from roots and soil during May and July as RLN and *D. dipsaci* populations increased from early spring (March) to mid-summer (July). Moreover, the result of this study indicated that the nematodes population density tends to decrease from the late summer (August) to the end of winter and start to increase from the early spring (March) to the mid-summer. Generally, the data results indicate that the population density of nematodes were the lowest in experiment plots during January and February and was the highest in plots between June and July (Table 22, 25 and 28). It was estimated that the population density of nematodes was increased in the early spring (March) plots than the first sampling results in 100gm of soil in the mid-summer (July). Between reproduction factors, soil temperatures showed a positive correlation with population densities of nematodes in this study. It was observed that the initial nematode population densities of nematodes

during both growing seasons. Moreover, nematodes multiplication was positively correlated with final population density and reached towards final densities $P < 0.001$, while there was a negative correlation time of sowing (Autumn and Spring) with initial nematode population in either Sanliurfa or Ankara.





6. CONCLUSIONS AND RECOMMENDATIONS

This study showed that there was a big species difference in nematode susceptible. *C. echinospermum* was more resistant to *P. thornei* and *D. dipsaci*, but more susceptible to *P. neglectus* than *C. arietinum* and *C. reticulatum*. Also, wilds cicer spp (*C. reticulatum* and *C. echinospermum*) were more resistant to nematodes than domestic (*C. arietinum*). This finding can be used to advantage in fields infested with these nematodes by selecting cultivars with the best levels of partial resistance. However, evaluation of advanced newly released cultivars and breeding lines is required for growers to use an advantage from such information in selecting the more resistant cultivars where needed. This study also indicated that better levels of resistance to both root lesion (*P. thornei*, *P. neglectus*) and *D. dipsaci* nematodes are available from accessions of the species *C. reticulatum* and *C. echinospermum*. When this level of resistance to both root lesion and *D. dipsaci* is available in cultivars developed, chickpea will be a better grown by growers where needed. Also, in this study showed that the laboratory study compared with the field study was very helpful, the laboratory study was confirmed result of field study in this study. A tiny subset of the collection was evaluated in the field experiment, where nematode populations were smaller than those recorded in the laboratory experiment and indicated there was a correlation between susceptible and resistant lines identified in field and laboratory experiment.



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CURRICULIM VITAE

He was born in 1985 in the city of Urmia in western Iran province of Azerbaijan. He speaks Azeri Turkish as the main language and he can speak English, Istanbul Turkish, Arabic, Germany and Persian. Also, he spent his life in Urmiye. It started middle school and high school in 1999 and 1996. He graduated from high school in 2002. He started college & University in Azad University, Faculty of Agriculture, Department of Crop Science, Khoy in 2002. Then, he started to master on agriculture and agrarian reform (MAAR) in the same university in 2008 and was completed his high degree of " THE IMPACT OF DEPTH AND PLANT BUNK ON THE WORK OF OIL SUNFLOWER ".

In 2012 he started study doctoral degree at the Faculty of Agriculture, Department of Plant Protection on plant nematology in Çukurova University in Turkey. There are many national and international scientific series which have been published in various conferences in Turkey and abroad by making many porpoises based on nematodes.