

Orijinal araştırma (Original article)

Distribution of nematodes on onion and their relationship with soil physicochemical characteristics in Karaman province, Turkey^{1,2}

Türkiye Karaman ilinde soğan ekiliş alanlarında bulunan nematodların dağılımı ve toprak fizikokimyasal özellikleri ile ilişkileri

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Summary

The distribution of plant-parasitic and free-living nematodes on onion rhizospheres and plant material and their relationship with soil physicochemical properties was investigated in Karaman province. Plant and soil samples totalling 100 were collected from onion fields during July, 2012. Nematode population density per three plants and 100 g of dry soil were determined for each sample. *Ditylenchus dipsaci* (Kühn 1857) (Tylenchida: Anguinidae) was found in 15% of plants and 61% of soil samples. Nematode numbers ranged between 0 and 140 (mean: 5) nematodes/three plants and 0–165 (mean: 33) nematodes/100 g dry soil. Other abundant plant-parasitic nematode genera were *Paratylenchus* (Micoletzky 1922) (Tylenchida: Paratylenchidae) (56%) and *Tylenchus* (Bastian 1865) (Tylenchida: Tylenchidae) (49%). The most abundant free-living nematodes were bacterivorous nematodes, which were found in 98% of samples, and were dominated by the *Cephalobus* (Bastian 1863) (Rhabditida: Cephalobidae) and *Eucephalobus* (Steiner 1936) (Rhabditida: Cephalobidae) genera. The majority of soil samples (68%) contained fungivorous nematodes belonging to the *Aphelenchus* (Bastian 1865) (Aphelenchida: Aphelenchidae) and *Aphelenchoides* (Fischer 1894) (Aphelenchida: Aphelenchoididae) genera. Omnivorous nematodes in the Dorylaimida order were found in 23% of soil samples. The distribution of plant- and bacterial-feeding nematodes were negatively related to the soil silt content and positively related to the soil sand content. Hyphal-feeding nematodes were negatively related to soil organic matter.

Keywords: nematode, onion, soil physicochemical characteristics, Karaman

Özet

Soğan ekiliş alanlarındaki bitki paraziti ve serbest yaşayan nematodlar tespit edilmiş ve toprak fizikokimyasal özellikleriyle ilişkileri araştırılmıştır. Yüz soğan ekili alandan 2012 Temmuz ayında 100 adet bitki ve toprak örneği alınmıştır. Nematod sayıları 3 bitki başına ve 100 g kuru topraktaki nematod sayısı olarak verilmiştir. *Ditylenchus dipsaci* (Kühn 1857) (Tylenchida: Anguinidae) bitki örneklerinin %15'inde, toprak örneklerinin %61'inde bulunmuştur. Nematod sayıları 3 bitki başına 0-140 nematod, ortalama olarak 5 nematod, 100 g toprakta ise 0-165 arasında ortalama olarak 33 nematod olarak tespit edilmiştir. *Paratylenchus* (Micoletzky 1922) (Tylenchida: Paratylenchidae) (56%) ve *Tylenchus* (Bastian 1865) (Tylenchida: Tylenchidae) (49%) türleri yoğun olarak bulunan diğer bitki paraziti nematodlardır. Serbest yaşayan nematodlar içinde en yüksek %98 oranında bulunan bakterivor nematodlar içinde en fazla bulunan cinsler *Cephalobus* (Bastian 1863) (Rhabditida: Cephalobidae) ve *Eucephalobus* (Steiner 1936) (Rhabditida: Cephalobidae) dur. *Aphelenchus* (Bastian 1865) (Aphelenchida: Aphelenchidae) ve *Aphelenchoides* (Fischer 1894) (Aphelenchida: Aphelenchoididae) cinslerine ait fungivor nematodlar örneklerin %86'sında bulunmuştur. Dorylaimida takımına ait omnivor nematodlar, örneklerin %23'ünde bulunmuştur. Bitki paraziti ve bakterivor nematodların dağılımı toprak mil içeriği ile ters ve kum içeriği ile doğrusal bir ilişki içerisindedir. Fungivor nematodlar ise toprak organik madde yoğunluğu ile negatif ilişki göstermiştir.

Anahtar sözcükler: Nematod, soğan, toprak fizikokimyasal özellikleri, Karaman

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Introduction

The onion is a vegetable, which plays an important role in human nutrition. Turkey is currently the seventh largest onion producer after China, India, USA, Iran, the Russian Federation and Egypt (Anonymous, 2014a). During 2012, the total area under onion cultivation in Turkey was 63 000 ha and total annual production was 1 819 000 tonnes (Anonymous, 2014a). Most production occurs in the Amasya province, followed by Ankara, Hatay, Tokat, Eskisehir, Corum, Adana and Bursa provinces in the Central Anatolian, East Mediterranean, Middle Black Sea and Trace Regions. Climatic conditions are favourable for onion production in Karaman province, with a production area of approximately 1000 ha providing 12 657 tonnes of onions during 2012 in Karaman (Anonymous, 2014b).

In addition to physiological disorders, diseases and pests compromise onion production. Plant-parasitic nematodes cause between 8.8 and 14.6% annual global crop losses (Nicol et al., 2011). The stem and bulb nematode, *Ditylenchus dipsaci* (Kühn 1857) (Tylenchida: Anguinidae) is a considerable constraint on onion production in Turkey. *D. dipsaci* was first isolated from onion plants in the Central Anatolian region by Yüksel (1958). Saltukoglu (1974) recorded *D. dipsaci* on onion plants in the Istanbul province. *Ditylenchus dipsaci* was recently recorded in the Konya, Karaman and Nevsehir onion-growing areas (Oztürk, 1990). *D. dipsaci* is currently on the European and Mediterranean Plant Protection Organization (EPPO) quarantine A2 list (No: 174), and has a local distribution in EPPO countries (Anonymous, 2014c).

Ditylenchus dipsaci results in stunting, discolouration and curving of leaves, local lesions and misshapen bulbs in plants and correspondingly, results in yield losses and reduction in market value (Anonymous, 2011a). Economic yield losses were calculated to occur at concentrations of the nematode of ten or more per 400 cm³ soil (Seinhorst, 1956). Yield losses of 60–80% were recorded in soils heavily infested by *D. dipsaci* (Nickle, 1991). In addition, it has been shown that *D. dipsaci* infestation resulted in 5–100% yield loss of onion and bulbous plants in the Aegean region (Anonymous, 2011b). It has been reported that 54.09% of onion fields in the Suluova district of Amasya province were infected by *D. dipsaci*, and an average of 65% yield reduction was recorded (Mennan & Ecevit, 2002).

Ditylenchus dipsaci was reviewed to be distributed in both heavy and light soils, although a higher incidence of disease severity was associated with heavy soils (Seinhorst, 1956; Miyagawa & Lear, 1970; Elgin et al., 1975; Sikora & Fernandez, 2005). Movement and dispersal of *D. dipsaci* tend to be greater in sandy soils than in clay soils (Elgin et al., 1975). Gerasimow (1954) found that the activity of *D. dipsaci* juveniles was dependent on the soil texture and pH. Activity of the juveniles was found to be higher in sandy soils at pH 7 and in loamy soils at pH 5.

Information on the distribution of nematodes as well as the damage caused on host plants requires urgent revision. Therefore, the aim of this study was to investigate the distribution of nematodes in the onion growing area in Karaman, the potential damage they may cause to onion cultivation and their relationship with soil physicochemical characteristics.

Materials and Methods

Sampling

Plant and soil samples totalling 100 were collected from onion fields located 1–2 km apart in the central Karaman, Ayranci and Ermenek districts from June to July during 2012. Sampling locations were recorded by a global positioning system (GPS). Sampling was performed in a zigzag pattern by taking a soil core sample using a 2.5 cm diameter width auger of 20 cm depth every 15 footsteps in the field. An average of 2 kg (2 000 cc) of soil was collected in total from 15–20 cores taken from each field. Three onion plants were sampled from each field using the same pattern.

Soil physicochemical analysis

Soil samples were analysed for pH, electrical conductivity (EC), CaCO₃ and phosphorus (P) contents, organic matter and texture. PH and EC ($\mu\text{S}/\text{cm}$) analyses were conducted according to the 1:2.5 soil: water ratio methodology described by Richards (1954). The CaCO₃ content (%) was analysed using the Scheibler calcimeter (Caglar, 1949). The P content (mg/kg) of soil samples was determined using the Olsen methodology (Knudsen, 1975). Organic matter (%) was determined using the Walkley–Black method (Walkley, 1946). Sand, silt and clay contents (%) were fractionated using the Bouyoucos hydrometer methodology (Tüzüner, 1990).

Nematode extraction and identification

Nematodes were extracted from three plants and 100 g fresh soil from each sample using the 'Modified Baermann Funnel Technique' (Hooper, 1986a). Extracted nematodes were counted under a light microscope at 40 \times magnification in 50 μl nematode suspension. The total number of nematodes in a 1 ml whole sample was calculated by multiplying the number of nematodes in 50 μl by 20. The soil moisture of each sample was calculated from the weight difference between fresh soil and soil dried at 90°C for one night. Nematode numbers were presented in 100 g of dry soil considering the soil moisture of each sample. Nematode genera were grouped according to trophic habits as described by Yeates et al. (1993). Permanent slides of the plant parasitic nematodes were prepared from all available specimens according to Hooper (1986b). Identification of the specimens was performed according to morphological and morphometric characteristics.

Statistical analyses

Soil physicochemical characteristics and nematode numbers were analysed using distribution analysis. The mean, maximum and minimum values were calculated for all variables, and the frequency of the soil texture class was determined. ANOVA was used to determine the frequency and population densities of nematodes and the student's t-test was used to determine the statistical differences between the nematode genera and trophic groups. Regression relationships among soil physicochemical characteristics and soil nematode populations were investigated using multivariate correlation analysis.

Results and discussion

Physicochemical characteristics of soils

Soil pH of collected samples ranged between 6.69 and 7.88, with a mean of 7.60. EC of soils ranged from 70.70 to 274 $\mu\text{S}/\text{cm}$ with a mean of 146.71 $\mu\text{S}/\text{cm}$. Soil physicochemical characteristics were evaluated according to standard values for soil fertility (Anonymous, 2013). The soil reaction changed the pH from neutral to weak alkaline. No problematic salinity levels were detected in any of the samples. Most samples had a high CaCO₃ content (range: 0.88–67.8%, mean: 24.07%). The soil P content was relatively high in all samples, with the lowest and highest being 3.23 mg/kg and 212.2 mg/kg, respectively (mean: 27.26 mg/kg). Most of the samples had low organic matter content, with the highest and lowest being 5.04% and 0.29%, respectively, in sampled soils. Mean organic matter content of the soil samples was 2.21%. The highest values of clay, silt and sand fractions were 55.29%, 55.29% and 65.69% and the lowest values were 16.11%, 13.67% and 13.67%, respectively. Mean clay, silt and sand contents were 35.45%, 33.13% and 31.23%, respectively. Most of the samples were clay loam (48%) and clay soil (34%). The frequency of sandy clay loam soil was relatively low (18%).

Frequency and population densities of nematodes

Nematodes obtained from the onion-planting areas were of the orders Tylenchida (Thorne 1949), Aphelenchida (Siddiqi 1980), Dorylaimida (Pearse 1942) and Rhabditida (Chitwood 1933). Identified trophic groups were plant-parasitic, hyphal-feeding, bacterial-feeding and omnivorous nematodes. Bacterial and plant-parasitic nematodes were at the highest frequency and population densities in sampled soils. Hyphal-feeding nematodes were grouped as having medium occurrence and population density. The lowest occurrence and population densities were obtained for omnivorous nematodes ($P < 0.05$).

Plant-parasitic nematodes were obtained from all samples. The mean population density of plant-parasitic nematodes was 196 nematodes/100 g of dry soil (range: 21–687 nematodes/100 g of dry soil). The most frequently obtained plant-parasitic nematodes were *D. dipsaci*, *Tylenchus* spp. and *Paratylenchus* spp.

The isolation frequency of *D. dipsaci* was 61%, which was significantly higher among plant-parasitic nematodes ($P < 0.05$). The population density of *D. dipsaci* varied between 0 and 165 nematodes per 100 g of dry soil (mean: 33 nematodes/100 g of dry soil). *D. dipsaci* was found in 15% of plant samples, with a population density of between 0 and 140 nematodes/three plants (mean: four nematodes/three plants). Figure 1 shows the distribution of *D. dipsaci* in soil of Karaman province according to population densities in sampling points. The population densities of *D. dipsaci* in soil samples were very high, with more than 100 nematodes/100 g of dry soil at the central villages of Morcali and Cukurbag. This area is irrigated and cultivated intensively for onion cultivation, and consequently, the higher *D. dipsaci* population densities were expected. In contrast, Damlapinar, Kizilyaka, Bozkondak and Baskisla are areas of non-irrigated onion production, where *D. dipsaci* was found at low frequency and population densities.

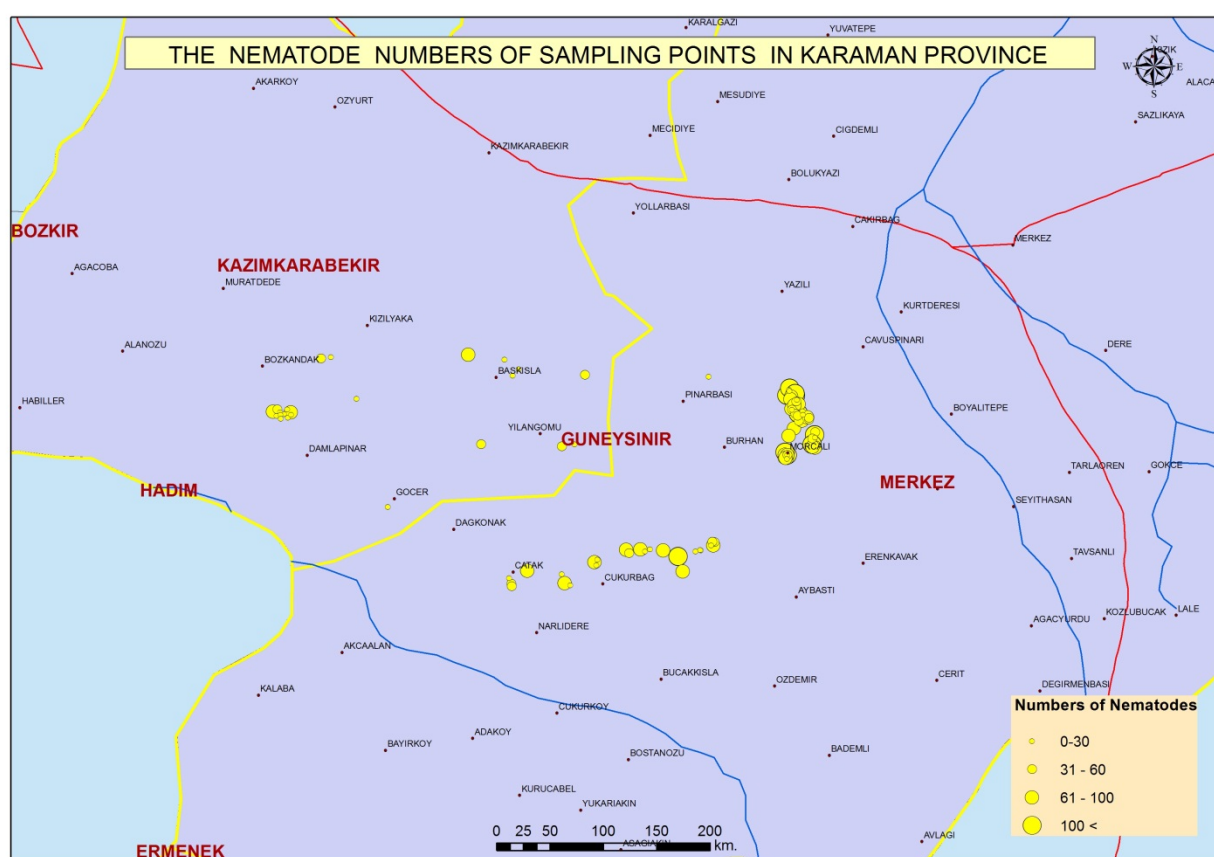


Figure 1. Distribution of *Ditylenchus dipsaci* based on population densities in soil in Karaman province during July 2012.

The second frequently identified genus belonging to plant parasitic nematodes was *Paratylenchus*, which was isolated from 56% of soil samples. The mean population density in sampled fields was 28 nematodes/100 g of dry soil, in a range of 0–163 nematodes/100 g of dry soil

Tylenchus spp. was found in 49% of collected soil samples. The population density of *Tylenchus* spp. ranged between 0–117 nematodes/100 g of dry soil (mean: 22 nematodes/100 g of dry soil).

Aphelenchus and *Aphelenchoides* are hyphal-feeding nematode genera that were found at a rate of occurrence of 74% and 76%, respectively, and their mean population densities were 65 (range: 0–355) and 79 (range: 0–335) nematodes/100 g of dry soil, respectively. There was no significant difference between population densities of hyphal-feeding nematode genera found. The occurrence of total hyphal-feeding nematodes was 86%, with a population range of 0–686 nematodes/100 g of dry soil (mean: 145 nematodes/100 g of dry soil).

The most abundant bacterial-feeding nematode genera was *Cephalobus* (86%; $P < 0.05$), followed by *Eucephalobus* (69%). The density of populations ranged between 0 and 446 nematodes/100 g of dry soil (mean: 81 nematodes/100 g of dry soil) and 0–2055 nematodes/100 g of dry soil (mean: 77 nematodes/100 g of dry soil) for *Cephalobus* and *Eucephalobus* genera, respectively. Total isolation frequency of bacterial-feeding nematodes was 98%, with population densities ranging between 0–2 105 nematodes/100 g of dry soil (mean: 223 nematodes/100 g of dry soil).

The frequency of omnivorous nematodes was 23% and populations ranged between 0 and 114 nematodes/100 g of dry soil (mean: 10 nematodes/100 g of dry soil).

Species identification of key plant-parasitic nematodes

Ditylenchus spp. includes migratory endoparasitic and hyphal-feeding nematodes (Yeates et al., 1993). Morphological identification of specimens obtained from plant samples showed that the specimens belonged to *D. dipsaci* species (Brzeski, 1991; Sturhan & Brzeski, 1991; Mollov et al., 2012). Morphometrical and allometrical measurements for the investigated specimens in comparison with the literature are presented in Table 1.

Table 1. Morphometric characteristics of *Ditylenchus dipsaci* collected from onion plant material. Measurements in μm and brackets indicate the mean \pm standard error (range) (isolate K44)

Characteristics	<i>D. dipsaci</i> (K44)	<i>D. dipsaci</i> (Brzeski, 1991)	<i>D. dipsaci</i> (Sturhan & Brzeski, 1991)	<i>D. dipsaci</i> (Mollov et al., 2012)
n	6	-	-	-
Total body length	1 203 \pm 49.41 (1156.8–1299.2)	-	(1 000–2 200)	-
Stylet	11.3 \pm 0.46 (11.2–12)	(10–12)	(10–13)	(11.5–12.3)
Anterior end to median bulb	65.86 \pm 7.53 (60.8–80)	-	-	-
Pharynx length	112.93 \pm 22.77 (108.8–168)	-	-	-
V	0.80 \pm 0.008 (0.8–0.82)	(0.76–0.86)	(0.76–0.86)	(0.79–0.81)
Post vulval uterine sac	0.40 \pm 0.02 (0.37–0.43)	(0.40–0.70)	-	-
T	81.6 \pm 1.75 (80–84.8)	-	-	(95–105)
a	37.85 \pm 4.48 (32–45.1)	-	(36–64)	(38–44)
b	9.72 \pm 1.24 (7.73–10.92)	-	(6.5–12)	(5.8–8.0)
c	14.40 \pm 0.97 (12.54–15.32)	(11–20)	(11–20)	(14–17)
C'	3.99 \pm 0.42 (3.57–4.81)	(3–6)	(3–6)	-

n: number of specimens, L: total body length, Stylet: stylet length, MB: median bulb length, OL: oesophagus length, V: (distance from anterior end of body to vulva)/body length, PUS: length of post vulval part of uterine sac in relation to vulva anus distance, T: tail length, a: body length/the largest width part of body, b: body length/distance from oesophagus intestine overlapping part to anterior end of body, c: body length/tail length, C': tail length/body width at anus,

Relationship between nematodes and soil characteristics

The total plant-parasitic and bacterial-feeding nematodes were negatively related to soil silt content ($R = 0.21$; 0.29 , respectively; $P < 0.05$). Individual nematode genera of *Ditylenchus*, *Tylenchus* and *Paratylenchus* additionally showed a negative correlation with soil silt content ($R = 0.20$ for all; $P < 0.05$) (Fig. 2).

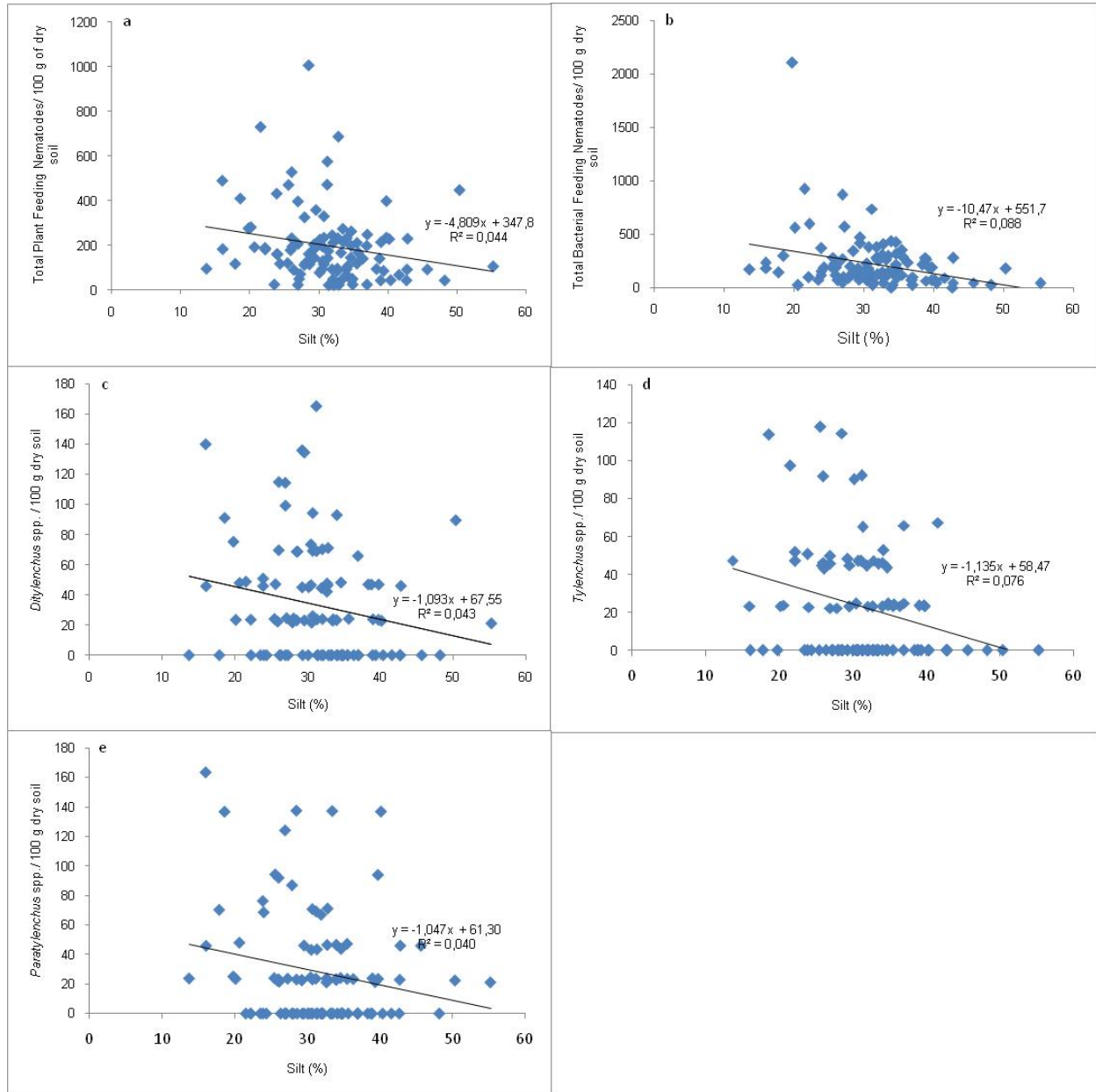


Figure 2. Relationship between soil silt content and plant- (a) and bacterial-feeding (b) nematode trophic groups, *Ditylenchus dipsaci* (c), *Tylenchus* spp. (d) and *Paratylenchus* spp. (e).

The total number of the bacterial-feeding nematodes was positively correlated to soil sand content ($R = 0.34$; $P < 0.05$). *D. dipsaci* and *Paratylenchus* spp. were positively correlated with soil sand content ($R = 0.19$ and $R = 0.22$, respectively; $P < 0.05$; Fig. 3). The distribution and survival of *D. dipsaci* in soil

has been found to be strictly dependent on soil texture and structure in past studies (Gerasimow, 1954; Miyagava & Lear, 1970; Elgin et al., 1975). Seinhorst (1954) reported that the distribution of *D. dipsaci* is circular and of a short distance in clay soils, whereas, in contrast, their distribution is irregular and of a relatively long distance in sandy soils, as presumably nematodes are carried by soil particles. The correlation analyses in the current study supported the previous findings for both *D. dipsaci* and plant-parasitic nematodes, with a positive relationship with soil sand content found. Even the effect of pH was not significantly related to nematode distribution in the current study, and *D. dipsaci* is known to be more active in alkaline and sandy soils as compared to acidic and loamy soils (Gerasimow, 1954). Miyagava & Lear (1970) had additionally noted that *D. dipsaci* had a longer infectivity in sandy soil containing 2.5% moisture.

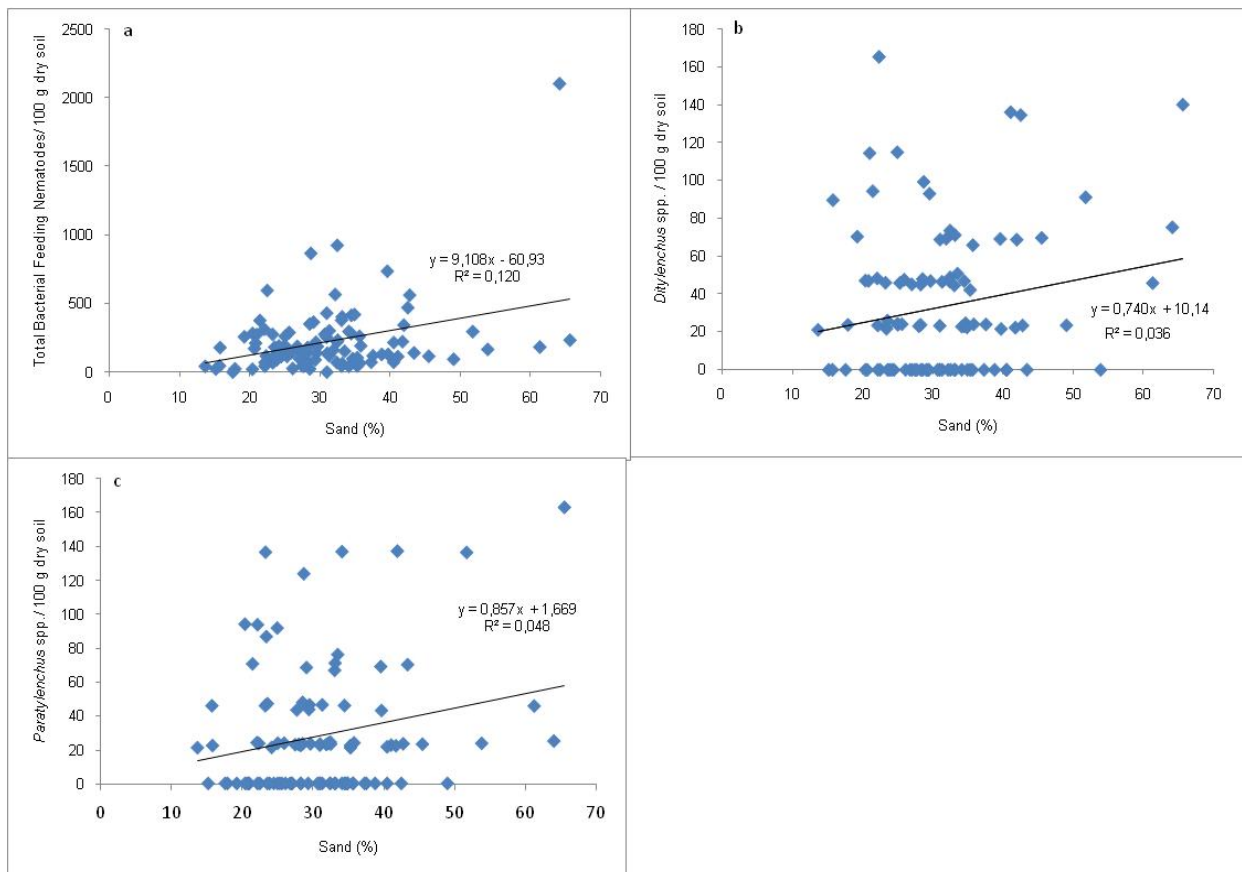


Figure 3. Relationship between soil sand content and bacterial-feeding nematodes (a), *Ditylenchus dipsaci* (b) and *Paratylenchus* spp. (c).

Hyphal-feeding and bacterial-feeding nematodes are indicators of soil microorganism abundance (Yeates et al., 1993; Bongers & Bongers, 1998). The increase in the population densities of *Aphelenchus* spp. and *Aphelenchoides* spp. are dependent on the mineralisation of organic matter in soil (Bongers & Bongers, 1998). Therefore, the results of the current study confirm this observation, with a significantly negative relationship between hyphal-feeding nematodes and soil organic matter content identified ($R = 0.26$; $P < 0.05$) (Fig. 4). High organic matter content, which is not degraded, caused lower multiplication of nematodes due to the lower microorganism abundance.

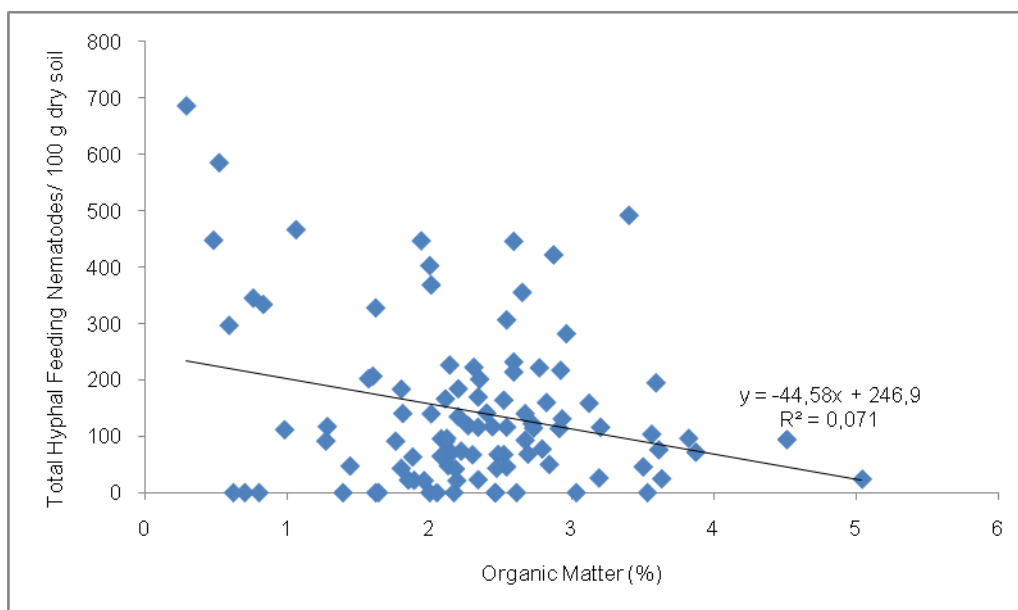


Figure 4. Relationship between soil organic matter content and hyphal-feeding nematodes.

The current study found that the main plant parasitic nematodes in onion-growing areas in Karaman were *D. dipsaci*, *Tylenchus* spp. and *Paratylenchus* spp. free living nematodes were in high incidence and abundance in the sampled areas. The distribution and abundance of nematodes was dependent on soil texture and mineralisation capacity. A wider nematode distribution and higher incidence was recorded in sandy and higher mineralised soils.

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