Original article (Orijinal araştırma)

Determination of the host status of some plant species with four different garlic populations of Ditylenchus dipsaci (Kühn, 1857)

Filipjev, 1936 (Tylenchida: Anguinidae)1

Bazı bitki türlerinin Ditylenchus dipsaci (Kühn, 1857) Filipjev, 1936 (Tylenchida: Anguinidae)’nin dört farklı sarımsak popülasyonuna karşı konukçuluk durumlarının belirlenmesi

Abstract

Stem and bulb nematode, Ditylenchus dipsaci (Kühn, 1857) Filipjev, 1936 (Tylenchida: Anguinidae) is widely distributed in areas where garlic is grown commercially in Turkey. One of the suitable methods for control of D. dipsaci under field conditions is rotation with non-host plant species. Thus, it is necessary to determine the host status of the plant species that can be used in rotation with the garlic plant. For this purpose, the host status of eight different plant species for four D. dipsaci populations obtained from important garlic growing areas was investigated in 2017 and 2018. The experiments were conducted with four replicates of treatments with nematode and without nematodes in a control environment room. Each plant was inoculated with 200 nematodes of the respective population. Six weeks after inoculation, the final nematode population in the plants and reproduction factors were determined. For all nematode populations, daffodil was an excellent host with reproduction factor (Rf) of 5.0-6.2. Onion and garlic plants were good hosts with Rf of 3.2-3.8 and 2.1-2.5, respectively. Lucerne, chickpea, leeks, lettuce and wheat were determined to be non-host species with Rf 0.6-0.7, 0.5-0.8, 0, 0 and 0.3-0.5, respectively. It was concluded that these non-host plant species can be used as rotational crops in the garlic production areas infested with D. dipsaci.

Keywords: Host, plant parasitic nematode, race, rotation, stem and bulb nematode

Öz

Soğan sak nematodu, Ditylenchus dipsaci (Kühn, 1857) Filipjev, 1936 (Tylenchida: Anguinidae) Türkiye’de ekonomik olarak sarımsak yetiştirciliği yapılan üretim alanlarında yaygın olarak bulunmaktadır. Ditylenchus dipsaci’nin tarla koşullarında mücadeledeki uygun yöntemlerden birisi de konukçu olmayan bitki türleri ile rotasyon uygulamalarıdır. Bu nedenle sarımsak bitikisi ile rotasyonla girebilecek bitki türlerinin nematodada konukçuluk durumlarının belirlenmesi önem arz etmektedir. Bu amaçla çalışmada seçik farklı bitki türünün önemli sarımsak yetiştirime alanlarının elde edilen dört farklı D. dipsaci popülasyonuna karşı konukçuluk durumları 2017-2018 yıllarında araştırılmıştır. Denemeler iki ilkim odası şartlarında nematodlu ve nematoduz bir bitkiler için dört tekrarlı olarak yürütülmüştür. Nematodlu bitkileri bitkinin başına 200 nematod inokule edilmiştir. İnokulasyondan altı hafta sonra bitkilerdeki sonuç nematod popülasyonun belirlenerek üreme faktörleri belirlenmiştir. Çalışmada bütün nematod popülasyonları için, nergis bitkisi 5.0-6.2 arasında üreme faktörü (Rf) ile mükemmel konukçu olarak belirlenmiştir. Soğan ve sarımsak bitikileri sırasıyla 3.2-3.8 ve 2.1-2.5 Rf ile iyi konukçu olarak tespit edilmiştir. Yonca, nohut,pirasa, marul ve buğday bitki türleri sırasıyla 0.6-0.7, 0.5-0.8, 0, 0 ve 0.3-0.5, Rf değerleri ile konukçu olarak belirlenmiştir. Gerçekleştirilen bu çalışma bu bitki türlerinin soğan sak nematodundan bulaşık olan üretim alanlarında rotasyon bitikisi olarak kullanılabileceği sonucuna varılmıştır.

Anahtar sözcükler: Bitki parazit nematod, irk, konukçu, münavebe, soğan-sak nematodu

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Introduction

Garlic is an economically important commodities in Turkey and worldwide. The main nematode constraint for garlic production is stem and bulb nematode, *Ditylenchus dipsaci* (Kühn, 1857) Filipjev, 1936 (Tylenchida: Anguinidae). Infected garlic plants show stunting, yellowing of leaves and shoots, deformation and abnormal cell growth in leaves and stems, lesions range from yellow to dark brown in the bulbs. The nematode reduces product quality and causes economically significant yield loses. In onion and bulbous ornamental plants, 5-100% damage can occur due to *D. dipsaci* (Duncan & Moens, 2006). *Ditylenchus dipsaci* is widely distributed in areas with temperate climates (Abd-Elgawad & Askary, 2015) and is reported from most of the onion and garlic production areas of Turkey (Mennan, 2001; Yavuzaslanoglu et al., 2019; Ocal, 2021).

*Ditylenchus dipsaci* has the greatest intraspecific difference in the host range of plant parasitic nematodes and therefore has the greatest number of synonyms with thirteen nominal species (Subbotin et al., 2005). The classification of this nematode is made at the race level according to the host status of the plants. There are more than 30 races that can multiply on economically important plant species (Sturhan & Brzeski, 1991). Seinhorst (1957) identified 11 different races of *D. dipsaci* using nine different plant species. Accordingly, he reported that onion [*Allium cepa* L. (Asparagales: Amaryllidaceae)], garlic [*Allium sativum* L. (Asparagales: Amaryllidaceae)] and pea [*Pisum sativum* L. (Fabales: Fabaceae)] were among the hosts of the onion race. Thorne (1961) reported that the hosts of the onion race of *D. dipsaci* were rice [*Oryza sativa* L. (Poales: Poaceae)], hyacinth [*Hyacinthus orientalis* L. (Asparagales: Asparagaceae)], daffodil [*Narcissus* spp. L. (Asparagales: Amaryllidaceae)], thistle [*Silybum marianum* (L.) Gaertn. (Asterales: Asteraceae)] and parsley [*Spinacia oleracea* L. (Caryophyllales: Amaranthaceae)]. Eight races of *D. dipsaci* were defined by Janssen (1994) according to the plant from which they were obtained. The races determined by Janssen (1994) were lucerne [*Medicago sativa*, L. (Fabales: Fabaceae)], red clover [*Trifolium pratense* L. (Fabales: Fabaceae)], oat [*Avena sativa* L. (Poales, Poaceae)], rye [*Secale cereale* L. (Poales, Poaceae)], sugar beet [*Beta vulgaris* L. (Caryophyllales: Amaranthaceae)], daffodil, tulip [*Tulipa* spp. L. (Liliales: Liliaceae)] and onion. Shubina (1992) reported that the onion race of *D. dipsaci* did not feed on maize [*Zea mays* L. (Poales: Poaceae)] but fed and reproduced on rice and pea. The host status of *D. dipsaci* obtained from onion in Amasya Suluova District in Turkey was investigated by Mennan (2001). Yavuzaslanoglu & Aksay (2021) reported susceptibility of plant species to onion and garlic populations of *D. dipsaci* from the Central Anatolia Region in Turkey. Viglierchio (1971) reported that the host status of local populations may be different. Therefore, the hosts of nematode populations that are distributed in different locations should be determined. However, the host status of different plant species to *D. dipsaci* populations obtained from different garlic production areas where most of the garlic production is undertaken has not been investigated widely in Turkey.

Therefore, the aim of this study was to investigate the host status of eight plant species using four *D. dipsaci* populations from garlic grown in production areas of Turkey.

Materials and Methods

Nematode populations

*Ditylenchus dipsaci* populations were collected in 2017 and 2018 from Adiyaman, Gaziantep, Kahramanmaras and Kastamonu Provinces, Turkey, in areas with intensive garlic production. Nematode populations were identified as *D. dipsaci* (Ates Sonmezoglu et al., 2020). Location information about *D. dipsaci* populations is given in Table 1.
Mass production of pure cultures of *Ditylenchus dipsaci* populations

Stem and bulb nematodes obtained from the samples did not contain sufficient numbers to be used directly and were not pure populations. Therefore, pure cultures of *D. dipsaci* populations were propagated by the carrot culture method using nematodes obtained from plant samples. Sterile carrot discs were prepared in 2017-2019 in Atatürk Horticultural Central Research Institute, Yalova, Turkey (Chitambar, 2003; Kühnhold et al., 2006). Firstly, the carrots that were washed in tap water, drained and peeled then placed 97% ethanol for 10-15 min. Then carrots were peeled again with a sterile knife, sliced into ~1 cm thick disc and placed individually in Petri dishes. One female and one male *Ditylenchus dipsaci* were transferred to each sterile carrot disc. Cultures were incubated at 19-20°C in the dark. Discs were cut into small pieces and placed on fresh sterile carrot discs for 2-4 months to maintain the cultures for use as inoculum (after extraction) for host status determination.

### Table 1. Geographic locations of *Ditylenchus dipsaci* populations

| Populations | Region         | Province       | District | Village   | Latitude         | Longitude       |
|-------------|----------------|----------------|----------|-----------|------------------|-----------------|
| ADY1        | South East Anatolia | Adıyaman | Tut      | Yeşilyurt | 37º44'55.55"N | 38º01'08.55"E  |
| GAZ4        | South East Anatolia | Gaziantep | Oğuzeli | Koçaklar  | 36º52'57.73"N | 37º31'57.40"E  |
| KAH2        | Mediterranean   | Kahramanmaraş | Pazarçık | Narlı/Karaçay | 37º22'13.96"N | 37º07'54.63"E  |
| KAS9        | Black Sea      | Kastamonu     | Taşköprü | Vakıfbelören | 41º30'07.37"N | 34º15'01.19"E  |

### Host status experiment

The plant species included in the experiment were wheat, lettuce, daffodil, chickpea, leek, garlic, onion and lucerne (Table 2).

### Table 2. The cultivars and sources of the plant species used for host status determination

| Plant species       | Cultivar name | Source                                           |
|---------------------|---------------|--------------------------------------------------|
| Chickpea (*Cicer arietinum* L.) | Azkan         | Altat Agriculture, Çorum, Turkey                 |
| Daffodil (*Narcissus tazetta* L.) | Karaburun     | Ege University, Department of Horticulture, İzmir, Turkey |
| Garlic (*Allium sativum* L.)       | Taşköprü 56   | Atatürk Horticultural Central Research Institute, Yalova, Turkey |
| Leek (*Allium porrum* L.)          | İnegöl 92     | Atatürk Horticultural Central Research Institute, Yalova, Turkey |
| Lettuce (*Lactuca sativa* L.)      | Grise maraichere | Atatürk Horticultural Central Research Institute, Yalova, Turkey |
| Lucerne (*Medicago sativa* L.)     | Bilensoy      | Intfa Agriculture, Konya, Turkey                 |
| Onion (*Allium cepa* L.)           | Kantartopu 3  | Atatürk Horticultural Central Research Institute, Yalova, Turkey |
| Wheat (*Triticum aestivum* L.)     | Flamura 85    | Altınbaşak Seed, Edirne, Turkey                  |

Experiment was conducted in a controlled environment room at the Atatürk Horticultural Central Research Institute, Yalova, Turkey in 2019. In the experiment, sand, field soil and farm manure were sterilized, mixed in a ratio of 70:29:1 and added to 12.5 x 12.5 x 20 cm pots (2.5 L). One seed of each plant species was planted per pot. Four weeks after planting, when the plants were at the three- to four-leaf stage, 10 µl of 1% carboxymethyl cellulose solution containing 200 nematodes was dropped at the leaf base of each plant (Kühnhold et al., 2006). Non-inoculated plants of each cultivar were used as controls. The pots were arranged in a completely randomized design with four replicates and plants were grown at 20-25°C and 70-80% RH in a 16:8 h L:D photoperiod. Six weeks after inoculation, plants were harvested and the plant growth parameters (plant height, stem diameter, number of leaves, root length, and combined shoot and root fresh weight) were measured. To extract nematodes, inoculated plants were cut into 1 cm pieces and placed in 15-cm Petri dishes according to a modified Baermann funnel technique for 48 h (Hooper et al., 2005). The extracted nematodes were counted under a stereomicroscope. The reproduction
factor (Ri), calculated as the number of nematodes obtained per plant at harvest divided by the 200 nematodes initially inoculated to the plant, was used to determine the host status of the test plants. Plant species were categorized as non-host with Ri < 1, poor host with 1 ≤ Ri < 2, good host with 2 ≤ Ri ≤ 4 and excellent host with Ri > 4 (Hajijahassani et al., 2016).

Statistical analysis

One-way analysis of variance was applied to the values of D. dipsaci ADY1, GAZ4, KAH2 and KAS9 populations in wheat, chickpea, daffodil, garlic, onion and lucerne. Differences between the treatments were evaluated using Tukey test at P ≤ 0.05. Comparison biplot analysis was conducted to determine the relationship between D. dipsaci populations and hosts in terms of reproduction factors of nematode populations on host plants. Differences in plant parameters between nematode inoculated and uninoculated treatments for each plant species with each nematode population were compared by t-test. Statistical analyses were performed using JMP (13th ed.) and GenStat (14th ed.) software.

Results

No nematodes were extracted from any inoculated lettuce and leek plants at harvest. However, nematodes were obtained from chickpea, daffodil, garlic, lucerne, onion and wheat plants. Ri values of all D. dipsaci populations ranged between 0.5-0.8 with chickpea, 5.0-6.2 with daffodil, 2.1-2.5 with garlic, 0.6-0.7 with lucerne, 3.2-3.8 with onion and 0.3-0.5 with wheat (Table 3).

Population-host interaction was statistically significant (F = 1.9, sd = 7.21, P < 0.05). Daffodil plants (Ri = 5.3) were rated as excellent hosts (Ri > 4) whereas onion (Ri = 3.4) and garlic (Ri = 2.3) were rated as good hosts for all nematode populations (2 ≤ Ri ≤ 4). Chickpea, leek, lettuce, lucerne and wheat plants were non-hosts for all nematode populations (Ri < 1). The average Ri of the populations was between 0.3 and 0.8 for chickpea, lucerne and wheat, while no nematode was extracted from lettuce and leek (Table 3).

Table 3. Reproduction factor for Ditylenchus dipsaci in different plant species in a pot experiment conducted in a growth room

| Ditylenchus dipsaci populations | Plant species |
|--------------------------------|--------------|
| Chickpea | Daffodil | Garlic | Leek | Lettuce | Lucerne | Onion | Wheat |
| ADY1 | 0.7±0.1Ad1 | 5.0±0.9Ba | 2.2±0.3Ac | 0.6±0.0 Ad | 0.6±0.0Ae | 0.6±0.0Ad | 3.3±0.3Ab | 0.5±0.1Ad |
| GAZ4 | 0.5±0.0Ad | 5.2±0.4Ba | 2.3±0.7Ac | 0.6±0.0 Ad | 0.6±0.0Ae | 0.6±0.0Ad | 3.2±0.4Ab | 0.4±0.1Ad |
| KAH2 | 0.8±0.1Ad | 5.0±0.5Ba | 2.5±0.5Ac | 0.7±0.0 Ae | 0.7±0.0Ae | 0.7±0.2Ad | 3.4±0.4Ab | 0.3±0.1Ade |
| KAS9 | 0.6±0.2Ad | 6.2±0.4Aa | 2.1±0.9Ac | 0.6±0.0 Ad | 0.6±0.0Ae | 0.7±0.2Ad | 3.8±0.5Ab | 0.5±0.1Ad |

1 Data are means of four replicates ± standard deviation. Means followed by the same lowercase letter within rows (plant species) or the same uppercase letters within columns (nematode populations) are not significantly different (P < 0.05, Tukey test).

No statistically significant differences between Ri values for nematode populations were found except for daffodil. In the daffodil, Ri of the KAS9 population was higher than other populations (P < 0.05).

The relationship between nematode populations and plant species was explained by comparison biplot analysis with a rate of almost 100% (Figure 1). The features close to the middle horizontal axis (PC1) were stable, while the stability of the features moving away from the axis was low. Also, the further a feature is located from the vertical axis (PC2) towards the right side (in the direction of the arrow) of the graph the stronger the relationship, and relationships are weaker towards the left side of the axis. According to the biplot, all the nematode populations examined formed a group. The stability of Ri on plant species of the ADY1 and GAZ4 nematode populations was greater (Figure 1). The biplot analysis showed that the stability of the onion plant was higher and the stability of the daffodil and garlic plants was lower. Chickpea, lucerne and wheat, with low Ri, and lettuce and leek plants with no reproduction were grouped together. The stability of lucerne plant was found to be higher compared to chickpea and wheat.
Most of the plant growth parameters for daffodil were significantly lower with inoculation compared to the controls for the different populations of *D. dipsaci*. Plant height was not adversely affected by the presence of KAH2 whereas there was significant reduction with the ADY1, GAZ4 and KAS9 populations (Table 4). Similarly, root height was significantly reduced by ADY1 and GAZ4 populations (Table 4).

Table 4. Percentage change in plant growth parameters in plant species inoculated with four *Ditylenchus dipsaci* populations

| Plant species | Nematode population | Shoot length | Number of leaves | Shoot diameter | Plant fresh weight | Root length | Number of roots |
|---------------|---------------------|--------------|-----------------|----------------|-------------------|-------------|----------------|
| Chickpea      | ADY1                | -14.0*       | -13.0           | -22.2          | -37.1             | -33.9       | -25.9          |
|               | GAZ4                | -2.8         | 0.0             | 5.5            | -11.4             | -53.9       | -30.1          |
|               | KAH2                | -20.3        | -13.0           | -38.8*         | -34.3             | -34.5       | -24.9          |
|               | KAS9                | -19.7        | -56.5*          | -38.8          | -40.0             | -51.5       | -22.3          |
| Daffodil      | ADY1                | -23.3*       | -15.5           | -44.9*         | -57.7*            | -30.5*      | -50.7*         |
|               | GAZ4                | -30.2*       | -4.4            | -31.2*         | -57.1*            | -40.6*      | -46.5          |
|               | KAH2                | -5.2         | -4.4            | -25.7*         | -43.3*            | -4.5        | -33.4          |
|               | KAS9                | -22.9*       | -11.1           | -34.8*         | -57.4*            | -33.7       | -50.7*         |
| Garlic        | ADY1                | -19.6        | -11.4           | -30.8          | -25.0             | -37.2       | -46.4*         |
|               | GAZ4                | -29.8        | -11.1           | -30.8*         | -58.3             | 23.3        | -38.7          |
|               | KAH2                | -23.5*       | -15.5           | -38.5          | -50.0             | -37.2*      | -40.5          |
|               | KAS9                | -25.8*       | -22.2           | -30.8          | -75.0*            | -44.2*      | -58.3*         |
| Leek          | ADY1                | -16.9*       | -22.4           | -36.6          | -50.0             | 13.3        | -39.8          |
|               | GAZ4                | -12.9        | -22.4           | -33.3           | -54.2*            | -20.0       | -31.0          |
|               | KAH2                | -11.9        | -22.4           | -23.3           | -41.6             | -20.0       | -31.0          |
|               | KAS9                | 3.3          | -8.6            | -20.0           | -16.6             | 13.3        | -8.8           |
| Lettuce       | ADY1                | 11.7         | -3.9            | -18.8           | -35.3             | -4.2        | 40.0           |
|               | GAZ4                | -16.9        | -5.8            | -8.3            | -29.8             | 9.9         | 58.4           |
|               | KAH2                | -14.8        | -12.5           | -10.4           | -45.3             | 2.8         | -9.6           |
|               | KAS9                | -1.64        | -7.8            | 10.4            | -5.8              | 2.8         | 46.4           |
| Lucerne       | ADY1                | -25.7*       | -10.7           | -40.0           | -40.6             | -25.4       | -28.0          |
|               | GAZ4                | -11.9*       | -18.5           | 10.0            | -21.8             | -25.4       | -37.6*         |
|               | KAH2                | -19.2        | -41.5           | -20.0           | -43.7             | -2.9        | -20.0          |
|               | KAS9                | -7.7         | -41.5           | -10.0           | -12.5             | -12.3       | -36.0          |
| Onion         | ADY1                | -5.0         | -3.3            | -14.9           | -37.4             | -27.0       | -15.7          |
|               | GAZ4                | -8.3         | -20.0           | 8.5             | -23.1*            | 9.0         | 6.1            |
|               | KAH2                | 13.1         | 0.0             | -10.6           | -35.2*            | -5.8        | -25.2          |
|               | KAS9                | -8.9         | 8.3             | 12.7            | -19.2             | 3.7         | -5.7           |
| Wheat         | ADY1                | 8.5          | 23.6            | -20.0           | -29.3             | -12.3       | -33.5          |
|               | GAZ4                | 16.1         | 36.4            | 33.3            | -21.9             | -33.8       | -41.6          |
|               | KAH2                | 21.4         | 0.0             | -20.0           | -17.0             | -4.6        | 2.7            |
|               | KAS9                | 8.1          | 5.5             | -13.3           | -34.1*            | 7.7         | -28.1          |

*D Differences between inoculated and uninoculated plants are significantly different according to the t-tests (P < 0.05).

With all nematode populations, stem diameter and plant fresh weight was reduced in inoculated daffodil. Mean stem diameter and shoot fresh weight reduced statistically significantly in all populations (P < 0.05) (Table 4). Other significant lower plant growth parameters in inoculated daffodil plants were the number of roots with ADY1 and KAS9 populations (Table 4).
Also, populations for D. dipsaci studies for populations. The number of D. dipsaci fresh shoot in shoot weight of garlic was significantly lower in plants inoculated with KAS9 population (P < 0.05) (Table 4). Fresh weight of onion was significantly lower in plants inoculated with GAZ4 and KAH2 populations (P < 0.05) (Table 4).

Other significant changes in plant growth parameters with inoculation were lower stem diameter and number of leaves in chickpea with inoculation of KAH2 and KAS9 population, respectively. Although no nematode reproduction occurred in leek, shoot length (ADY1 population) and plant fresh weight (GAZ4 population) was found lower in inoculated plants (Table 4).

Mean shoot length of lucerne was significantly lower with inoculation with ADY1 and GAZ4 populations. Also, number of roots was significantly lower in nematode inoculated (GAZ4 population) lucerne plants (P < 0.05) (Tables 4).

**Discussion**

In this study, the host status of eight plant species of potential use in crop rotations for managing D. dipsaci in garlic was determined. Daffodil was found to be an excellent host, onion and garlic good hosts for D. dipsaci populations from garlic in South East Anatolia and Black Sea Regions in Turkey. In previous studies (Mennan, 2001; Yavuzaslanoglu & Aksay, 2021) similar results for D. dipsaci populations from other geographic regions of Turkey were obtained. However, Yavuzaslanoglu & Aksay (2021) did not obtain D. dipsaci reproduction daffodil, but it was found to be an excellent host in the current study. The reason for this could be the response of a different daffodil cultivar or difference in virulence of nematode populations applied. Whether this difference was due to the plant cultivar or nematode populations should be determined by investigating the host status of a range of daffodil cultivars to D. dipsaci populations. Also, in the current study, lower R_{hi} values were determined for onion and garlic plants than by Yavuzaslanoglu & Aksay (2021) and were classified as good hosts rather than excellent hosts.

In a recent study (Poirier et al., 2019) in Canada, lucerne and lettuce were found to be non-hosts of a garlic population of D. dipsaci, similar to our study. Also, consistent with the findings of the present study, Hajihassani et al. (2016) reported that wheat was a non-host, chickpea cultivars were poor hosts and garlic a good host.
*Ditylenchus dipsaci* populations have been shown to decrease significantly with 3-4 years of rotation with non-host plants (Hooper, 1984; Roberts & Grathead, 1986). It is essential to know the host range of the population of *D. dipsaci* in an area in order to successfully design a crop rotation strategy to manage *D. dipsaci*. According to our results, lucerne, chickpea, wheat, lettuce and leek are non-hosts for *D. dipsaci* and this host status was not affected by the nematode population applied. Therefore, these plants can be recommended as rotational plants in garlic areas infested with *D. dipsaci*.

Shoot length, stem diameter, root length, number of roots and leaves, and whole plant fresh weight properties were used for evaluation of effect of *D. dipsaci* on the plants tested. Paralleling nematode reproduction, several plant growth parameters were identified to be affected by nematode inoculation. The non-host plant species in this study were unaffected.

To continue this work, it is necessary to test the non-host plant species identified in this study under natural infestation of *D. dipsaci* in the field and to consider their economic and agronomic value as rotational crops.

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