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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

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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 (R_f) of 5.0-6.2. Onion and garlic plants were good hosts with R_f 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 R_f 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 yetiştiriciliği yapılan üretim alanlarında yaygın olarak bulunmaktadır. *Ditylenchus dipsaci*'nin tarla koşullarında mücadelesi için uygun yöntemlerden birisi de konukçu olmayan bitki türleri ile rotasyon uygulamalarıdır. Bu nedenle sarımsak bitkisi ile rotasyona girebilecek bitki türlerinin nematoda konukçuluk durumlarının belirlenmesi önem arz etmektedir. Bu amaçla çalışmada sekiz farklı bitki türünün önemli sarımsak yetiştirme alanlarından elde edilen dört farklı *D. dipsaci* popülasyonuna karşı konukçuluk durumları 2017-2018 yıllarında araştırılmıştır. Denemeler iklim odası şartlarında nematodlu ve nematodsuz bitkiler için dört tekerrürlü olarak yürütülmüştür. Nematodlu bitkilere bitki başına 200 nematod inokule edilmiştir. İnokulasyondan altı hafta sonra bitkilerdeki sonuç nematod popülasyonu belirlenerek üreme faktörleri belirlenmiştir. Çalışmada bütün nematod popülasyonları için, nergis bitkisi 5.0-6.2 arasında üreme faktörü (Rt) ile mükemmel konukçu olarak belirlenmiştir. Soğan ve sarımsak bitkileri sırasıyla 3.2-3.8 ve 2.1-2.5 Rt ile iyi konukçu olarak tespit edilmiştir. Yonca, nohut, pırasa, marul ve buğday bitki türleri sırasıyla 0.6-0.7, 0.5-0.8, 0, 0 ve 0.3-0.5, Rt değerleri ile konukçu olmayan bitki türleri olarak belirlenmiştir. Gerçekleştirilen bu çalışma ile konukçu olmayan bitki türlerinin soğan sak nematodunun bulaşık olan üretim alanlarında rotasyon bitkisi olarak kullanılabileceği sonucuna varılmıştır.

Anahtar sözcükler: Bitki paraziti nematod, ırk, 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 Adıyaman, Gaziantep, Kahramanmaraş 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.

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	Pazarcık	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 (<i>Medicago sativa</i> 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 (R_f), 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 $R_f < 1$, poor host with $1 \le R_f < 2$, good host with $2 \le R_f \le 4$ and excellent host with $R_f > 4$ (Hajihassani 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 $P \le 0.05$. 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. R_f 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 ($R_f = 5.3$) were rated as excellent hosts ($R_f > 4$) whereas onion ($R_f = 3.4$) and garlic ($R_f = 2.3$) were rated as good hosts for all nematode populations ($2 \le Rf \le 4$). Chickpea, leek, lettuce, lucerne and wheat plants were non-hosts for all nematode populations ($R_f < 1$). The average R_f 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).

Ditylenchus	Plant species								
<i>dipsaci</i> populations	Chickpea	Daffodil	Garlic	Leek	Lettuce	Lucerne	Onion	Wheat	
ADY1	0.7±0.1Ad ¹	5.0±0.9Ba	2.2±0.3Ac	0.0±0.0 Ad	0.0±0.0Ad	0.6±0.2Ad	3.3±0.3Ab	0.5±0.1Ad	
GAZ4	0.5±0.2Ad	5.2±0.4Ba	2.3±0.7Ac	0.0±0.0 Ad	0.0±0.0Ad	0.6±0.1Ad	3.2±0.4Ab	0.4±0.1Ad	
KAH2	0.8±0.1Ad	5.0±0.5Ba	2.5±0.5Ac	0.0±0.0 Ae	0.0±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	0.0±0.0 Ad	0.0±0.0Ad	0.7±0.2Ad	3.8±0.5Ab	0.5±0.1Ad	

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

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 R_f values for nematode populations were found except for daffodil. In the daffodil, R_f 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 R_f 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 R_f , 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).

Plant	Nematode	Shoot	Number of	Shoot	Plant	Root	Number o
species	population	length	leaves	diameter	fresh	length	roots
000000	ADY1	-10.0	-13.0	-22.2	-37.1	-33.9	-25.9
Chickpea	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*
	ADY1	-19.6	-11.4	-30.8	-25.0	-37.2	-46.4*
Garlic	GAZ4	-29.8	-11.1	-30.8*	-58.3	23.3	-38.7
Ganic	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*
	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
Leek	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
	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
Lettuce	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

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

* 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). Determination of the host status of some plant species with four different garlic populations of *Ditylenchus dipsaci* (Kühn, 1857) Filipjev, 1936 (Tylenchida: Anguinidae)

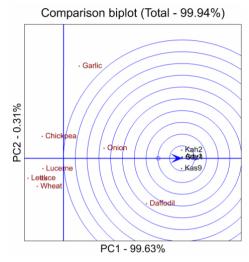


Figure 1. Biplot of reproduction factor of *Ditylenchus dipsaci* ADY1, GAZ4, KAH2 and KAS9 populations in chickpea, daffodil, garlic, leek, lettuce, lucerne, onion and wheat.

Garlic plant species had significantly lower plant growth parameters with nematode treatment. Mean shoot and root length decreased with KAH2 and KAS9 populations (P < 0.05) (Table 4). Number of roots in nematode inoculated plants with ADY1 and KAS9 populations was significantly lower (Table 4). Garlic shoot fresh weight 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 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_f 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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