Investigation of resistance of some pistachio cultivars against root-knot nematode, Meloidogyne javanica

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Abstract

Background: *Meloidogyne* species, also known as root-knot nematodes, are the most economically viable plant parasitic nematodes. The best method for controlling them is to use resistant cultivars. The present study examined the resistance of several pistachio cultivars against M. javanica.

Materials and methods: The seeds of five pistachio cultivars including Kale-Ghouchi, Badami-Sefid, Mumtaz, Kale-Bozi, and Kal-Khandan were obtained from the pistachio-growing regions of Iran. Then these seeds were planted in the part of the orchard that was infested with root-knot nematode in a randomized complete block design (RCBD) with three replications and at a distance of two meters from each other. By removing the seedlings from the soil a year after planting, the parameters of the gall index and the nematode population in the infected root were measured. The Taylor and Sasser approach was used to assess the evaluation's findings.

Results: The number of galls formed in the root was more than 30 galls and the index was more than 4 in all tested cultivars, therefore all tested pistachio cultivars were sensitive to root-knot nematode. The Badami-Sefid cultivar had the highest amount of gall production in the root with the production of more than 100 galls on the root compared to other cultivars

Conclusion: Considering the sensitivity of commercial cultivars, it is better to use cultural control and phytosanitary methods to prevent root-knot nematode damage. Certified seedlings (free of nematodes), suitable soil texture, and the use of root growth enhancers are recommended.

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1. Introduction

Pistachio (*Pistacia vera* L.) belongs to the *Anacardiaceae* family. *Pistacia* has 11 species, all of which secrete turpentine. The members of this dioecious genus have combed leaves and single-seeded drupe fruit, and its pollination is by wind [1]. Pistachio is one of the most important horticultural products of Iran and has an important contribution to the country's non-oil exports. The area under pistachio cultivation in 2019 was about 534 thousand hectares and its production was about 387 thousand tons [2]. Cultivation of this plant is widespread in 28 provinces and Kerman, Khorasan-Razavi, and Yazd provinces have the highest pistachio cultivation area with 212, 112, and 54 thousand hectares respectively [2]. Root-knot nematodes are economically the most important plant parasitic nematodes in the world they attack more than 2000 plant species and often establish a complex and specific nutritional relationship with their hosts in a wide range of plants and have caused a decrease of about five percent of the total production of agricultural products in the world [3, 4, 5]. Root-knot nematodes belong to the *Meloidogynidae* family and the *Meloidogyninae* subfamily [4]. This subfamily includes only the single genus *Meloidogyne*, which has more than 100 species. Of these 4 species are widely distributed and have economic importance in agriculture. It is rare to find a plant that does not host this genus. Global distribution, wide host range, and interaction with other plant pathogens in disease complexes make them as one of the five key pathogenic agents and the most important plant pathogens, which threaten the world's food supply [4]. Economically, the four species *Meloidogyne hapla*, *M. arenaria*, *M. javanica*, and *M. incognita* are responsible for more than 95% of the damage caused by root-knot nematodes to agriculture [5].

The species *M. incognita* has spread in a wider geographic range than other species (approximately from 40 degrees north latitude to 33 degrees south latitude). The average annual temperature suitable for the activity of this nematode is between 18 and 30 ºC, but the largest nematode population is seen at a temperature of 24 to 30 degrees. This species is mainly found together with *M. javanica* [6].

The species *M. javanica* has been found in all parts of the world and is the second most common species. This species is usually found in areas where the average annual temperature is between 18 and 38º C [7]. So far, in addition to the four main species of root-knot nematodes that have been previously reported from different regions of Iran. three other species, *M. cruciani*, *M. microcephala*, and *M. thamesi*, have been identified from several regions [8, 9, 10, 11, 12]. Studying the resistance of pistachio cultivars to plant pathogens, including nematodes, is necessary for the development of new orchards. The use of resistant cultivars or even with less sensitivity will reduce the problems of controlling subsequent diseases.

2. Materials and methods

2.1. Pistachio sampling

Seed samples of 5 cultivars were collected from important pistachio-growing areas of Iran located in Qazvin, Faizabad, and Rafsanjan. The collection time was done according to the season of pistachio fruiting and harvesting. The cultivars Kale-Ghouchi and Mumtaz were collected from the Rafsanjan region, the Badami-Sefid cultivar from the Faiz-Abad

region, and the cultivars Kale-Bozi and Kal-Khandan were collected from Qazvin. To preserve the physiological characteristics, the collected pistachio seeds were stored in the refrigerator until planting.

2.2. Soil inoculation with root-knot nematodes

To investigate the level of resistance of different pistachio cultivars to root-knot nematode, a plot of root-knot nematode contaminated field was selected. Contamination was confirmed by sampling and extracting nematodes from the root rhizosphere of different parts by centrifugation method [5]. Considering the abundance of *M. javanica* species in the region and the fact that the plot of the field was only infected with this species, in this project, the resistance of five pistachio cultivars was evaluated with this species. In the first year of the project, the contaminated plot of land was divided into 16 plots, and tomato (as a plant sensitive to root-knot nematode) was planted to increase the nematode population and provide sufficient inoculum. After the end of the growing season, samples were taken from tomato roots and the soil of cultivated plots to investigate the level of infection with root-knot nematodes and the distribution of nematodes in the soil of these plots, and the number of nematode galls was measured in a certain weight of the root. To prepare the soil sample, three soil samples were taken from each contaminated plot from a depth of 0 to 30 cm. After mixing three samples, one sample with a volume of 100 cm^3 was used for evaluation. The soil sample was examined using the Jenkins method [15] and the population density of juvenile (J2)/100 cm^3 was determined.

After the end of the tomato growing season (the end of the first year of the project implementation) and identifying the parts infected with nematodes, due to the insufficiency of the nematode population and the non-uniformity of the nematode in the soil, to make the contamination uniform and increase the nematode population. In the infected plot, in the second year of the project implementation, tomatoes were planted in 6 infected plots for another year, so that the nematode population in the tested plots reached a sufficient and appropriate level. At the end of the cropping season, the contaminated roots remaining in the ground were well mixed with the soil. As in the previous year, sampling was done from the soil of the infected plot to determine the nematode population (sampling was done from 4 different points of each plot (30 m^2), and a 250 cm^3 soil sample was selected from the mixture of these 4 samples of 500 cm^3 and the nematode population was determined in the soil of the plots as in the previous year.

2.3. Preparing the field for planting pistachio seeds

Before planting the pistachio seeds, to make the contamination more uniform in all parts of the field, the soil of the contaminated field was completely mixed to a depth of 30 cm in all the contaminated plots and spread again on the ground. Further, after the surface plowing of the contaminated field, the irrigation system, the necessary planning and forecasting were done to demarcate the parts and create the path of water movement. To investigate the resistance of pistachio cultivars to root-knot nematode, a completely randomized block design was used. For this purpose, after blocking the target plot, the seeds of cultivars were planted in the contaminated ground in three repetitions, each repetition included 10 pistachio seedlings. For this purpose, seeds of different pistachio

varieties were first soaked in water for 24 hours. To prevent the growth of fungi such as *Aspergillus*, the seeds were disinfected with fungicides such as PCNB and Captan. Then the pistachio seeds were placed between two layers of wet cloth. After a few days, the seeds started to germinate. After the roots of the pistachio seeds grew to a sufficient size, they were transferred to the field and planted in the soil at a depth of 3 to 4 cm.

During the cropping season, several operations (irrigation, fertilization, and weed control) were carried out. One year after planting, the tested seedlings were removed from the soil and evaluated. After the seedlings emerged from the soil, the roots of the seedlings were weighed and 5 grams of roots from each treatment were examined to determine the number of galls formed on the root and the number of nematode populations of different ages inside the root. Seedlings were evaluated based on the table proposed by Taylor and Sasser (1978) (Table 1).

2.4. Calculation of gall index and egg sac:

To count the number of galls, the roots of the infected seedlings were first gently washed under running water and then placed on filter paper to remove excess water. Then the roots were dyed for 10 to 15 minutes in Phloxin-B 5% color solution. Then, based on the proposed system of Taylor and Sasser (1978), the gall index was determined (Table 1).

2.5. Calculation of the population of root-knot nematodes inside the root

After counting the number of galls, each root was cut into one-centimeter pieces and crushed in a blender, and the nematodes inside the roots were extracted using the method of Van Bezooijen [17].

2.6. Evaluation of growth factors of pistachio cultivars

At the end of the growing season, after removing the pistachio seedlings from the soil, in addition to measuring the number of galls and the number of J2 root-knot nematodes, to evaluate the growth and development of the treated pistachio cultivars, their stem length, and weight of pistachio seedlings was also measured as growth factors. The numbers obtained from the test were related to each of the factors that were measured and analyzed, and the averages were compared by Duncan's multiple range test.2.7.

Table 1. Determining the reactions based on the number of galls and the gall and egg sac index based on the proposed system of Taylor and Sasser [16]

Reaction	Index	Number of galls or egg sacs
High resistant	0	0
Resistant	1	1-2
Semi resistant	2	3-10
Low resistant	3	11-30
Susceptible	4	31-100
Highly sensitive	5	>100

3. Results

Artificial contamination of seedling planting site: The amount of contamination with J2 root-knot nematodes was determined (Table 2), which was found to be suitable for the implementation of the project.

Table 2. The level of contamination of 1-6 pieces of land infected with second-stage juvenile (J2) of root-knot nematode (*Meloidogyne javanica*)

Population J2 in 250 milliliters of soil	Plot number
620	1
360	2
380	3
540	4
250	5
490	6

Evaluation of J2 population of root-knot nematode and gall index: By comparing the average number of galls produced on the root and the gall index in different cultivars and analyzing the data, the resistance of different pistachio cultivars to *M. javanica* nematode was determined. The degree of resistance calculated based on the gall index according to the proposed system of Taylor and Sasser [16] is shown in Table 5. The number of galls formed in the root was more than 30 galls and the index was more than 4 in all tested cultivars, therefore the reaction of all tested pistachio cultivars is sensitive to root-knot nematode. At the same time, among the tested cultivars, the Kale-Ghouchi variety had the least amount of gall production in the root, and the Badami-Sefid cultivar had the highest amount of gall production in the root with the production of more than 100 galls on the root compared to other cultivars (Table 3).

Table 3. Comparison of index averages and the number of galls in the root of different pistachio cultivars

Cultivar	Average gall number	Gall index
Badami-Sefid	>100	5
Mumtaz	>54	4
Kale-Bozi	>77	4
Kal-Khandan	>77	4
Kale-Ghouchi	>53	4

Population of Root-knot nematode J2: Table 4 shows the J2 population of *M. javanica* in 5 grams of roots of different pistachio cultivars. Among pistachio cultivars, the lowest amount of nematode population in the infected roots belongs to the Kale-Ghouchi variety, and

the highest amount of nematode population was also observed in the white almond variety.

Table 4. Comparison of the average population of the J2 knot nematode (*Meloidogyne javanica*) in the roots of different pistachio cultivars

Cultivar	Nematode population in 5 grams of root
Badami-Sefid	1837
Mumtaz	1211
Kale-Bozi	1405
Kal-Khandan	1028
Kale-Ghouchi	1120

Calculation of growth factors of pistachio seedlings: The results showed that the average stem weight and length in pistachio cultivars had a significant difference at the 5% level (Tables 5 and 6). The results show that all the tested pistachio cultivars were sensitive to the *M. javanica* nematode species. The variation in growth observed across different cultivars may be due to their underlying genetic and physiological characteristics. Therefore, relying solely on parameters like stem length and weight is not a valid way to assess the resistance of pistachio cultivars to M. javanica nematodes.

Table 5. Comparison of the average stem height of pistachio cultivars based on Duncan's multiple-range test

Cultivar	Average stem height (Cm)	Statistical grouping (α=0.05)
Mumtaz	16.44	A
Badami-Sefid	16.06	B
Kal-Khandan	14.19	C
Kale-Bozi	13.06	D
Kale-Ghouchi	12.56	E

Table 6. Comparison of the mean weights of the pistachio cultivars tested based on Duncan's multiple range test.

Cultivar	Average stem weight (gr)	Statistical grouping (α=0.05)
Badami-Sefid	5.25	A
Kale-Bozi	5.17	A
Mumtaz	4.8	B
Kale-Ghouchi	4.13	C
Kal-Khandan	3.14	D

4. Discussion

The use of resistant or tolerant pistachio cultivars is essential for managing plant-parasitic nematodes, especially considering the significant environmental pollution caused by nematicides and other chemicals [18]. Few studies have examined how various pistachio varieties react to root-knot nematodes. Most research has concentrated on identifying the parasitic nematodes found in pistachio orchards rather than assessing the cultivars' responses to them [19, 20]. In a survey of parasitic nematodes in California's pistachio orchards, several species of these nematodes have been identified; however, their presence is not considered a significant threat to pistachio production in the state [20]. Madani investigated the responses of both domestic and wild cultivars of pistachio to the *M. javanica* species and races two and four of *M. incognita*. The results indicated that the domestic varieties Ardakani, Qazvini, and Fandoghi resisted *M. incognita*. Additionally, the common Damghan variety demonstrated resistance to both *M. incognita* and *M. javanica* species. Furthermore, the Australian wild population also showed a resistant reaction to both *M. javanica* and *M. incognita* species [13].

Kargar-Bideh investigated how various wild species and native cultivars of pistachio respond to the root-knot nematode (*M. incognita*) in greenhouse conditions. The results indicated that domesticated pistachio rootstocks exhibited lower resistance to the nematodes compared to wild species. Notably, two specific pistachio species, Khinjuk and Bane, demonstrated a greater tolerance to nematodes than other cultivars [22].

The research results indicated that there was no significant difference in the level of resistance to infection among the tested pistachio cultivars. All cultivars with a resistance rating greater than four (as defined by Taylor and Sasser) were classified as susceptible to infection. Among the cultivars tested, the Kaleh Ghouchi cultivar exhibited the least gall production and the lowest nematode population in the roots, suggesting it has the highest resistance to the root-knot nematode *M. javanica* compared to the other cultivars.

5. Conclusion

The study was conducted on a limited number of pistachio cultivars. In addition to the cultivars tested in this research, it is advisable to investigate the responses of other important and commercially cultivated pistachio varieties to root-knot nematode species in future studies to identify sources of resistance. Utilizing a resistant or tolerant pistachio cultivar to root-knot nematodes when establishing a new orchard is crucial for ensuring the health of the trees during their productive years. The research findings indicate that all tested pistachio cultivars are sensitive to the root-knot nematode *M. javanica*. Therefore, it is recommended to select pistachio cultivars that exhibit lower sensitivity to this nematode when establishing new orchards or improving existing areas of pistachio cultivation. Additionally, implementing favorable practices such as maintaining phytosanitary conditions, using healthy seedlings, and enhancing soil quality can significantly contribute to increasing cultivar tolerance and minimizing nematode damage.

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Conflict of interest declaration

The authors state that there is no conflict of interest.

Code of ethics

In this research, no living entity has been used, and the research stages have been conducted in a laboratory.

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