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Original article (Orijinal araştırma)

Resistance of local okra cultivars against *Meloidogyne incognita* (Kofoid & White, 1919) (Nematoda: Meloidogynidae), effects of nematode infestation on growth parameters and leaf macro- micronutrients

Yerel bamya çeşitlerinin *Meloidogyne incognita* (Kofoid & White, 1919) (Nematoda: Meloidogynidae)'ya karşı dayanıklılığı, nematod enfeksiyonunun bamyanın morfolojik özelliklerine ve yapraklarda makro-mikro element içeriğine etkisi

Çetin NACAR[1*] **Adem ÖZARSLANDAN[2]**

Abstract

Okra is an important species of vegetable grown in Turkey and around the world. Root-knot nematodes cause serious yield losses in vegetables. Twenty-six cultivars okra were obtained from the Aegean Agricultural Research Institute in 2002. Resistance of this cultivars to nematode was determined and changes induced with nematode infestation on plant morphology and macro- and micronutrients in leaves measured. The experiments were conducted in completely randomized design with four replicates in climate-controlled glasshouse at Alata Horticultural Research Institute in 2019. The cultivars were tested against *Meloidogyne incognita* (Kofoid & White, 1919) (Nematoda: Meloidogynidae) at 25 ± 2ºC. Sixty days after inoculation, root gall indexes varied with an average of 4.56. Compared to uninoculated controls, nematode inoculated reduced plant height by 46.0%, stem thickness by 9.06%, root lengths by 39.7%, leaf widths by 15.1%, leaf lengths by 20.5% and increase root weight by 14.5%. Leaf macro- and micronutrients were determined. Compared to the control, nematode inoculation reduced leaf N, P, K and Mg concentrations by 2.31, 5.85, 5.24 and 10.3%, respectively, and increased Ca, Fe, Zn, Mn and Cu concentrations by 7.91, 17.0, 13.4, 118 and 15.6%, respectively.

Keywords: *Meloidogyne incognita*, morphological traits, nutrient, okra, resistance

Öz

Bamya dünyada ve ülkemizde yetiştiriciliği yapılan önemli sebze türlerinden birisidir. Kök-ur nematodları sebzelerde önemli ürün kayıplarına neden olmaktadır. Ege Tarımsal Araştırma Enstitüsü'nden 2002 yılında getirilen 26 yerel bamya çeşidinin nematodlara karşı dayanıklılık durumları, bitki morfolojisinde meydana gelen değişikliklere ve bitki yapraklarındaki makro-mikro besin elementleri içeriğine bakılmıştır. Deneme tesadüf parselleri deneme desenine göre 4 tekrarlamalı olarak Alata Bahçe Kültürleri Araştırma Enstitüsü Müdürlüğü'ne ait kontrollü cam seralarda 2019 yılında yürütülmüştür. Yerel bamya çeşitleri *Meloidogyne incognita* (Kofoid & White, 1919) (Nematoda: Meloidogynidae), popülasyonuna karşı 25 ± 2ºC'de testlenmiştir. İnokulasyondan sonra bitkiler 60. günde bitki sökümleri yapılarak kök gal indeksi değerlendirmesi yapılmış ve ortalama 4.56 ur indeksi oluşturduğu belirlenmiştir. Bitkisel özelliklerde kök-ur nematod uygulamasının kontrol bitkilerine göre bitki boyunu ortalama %46.0, gövde kalınlığını %9.06, kök uzunluğunu %39.7, yaprak eni uzunluğunu %15.1, yaprak boyu uzunluğunu %20.50 oranında azalttığı ve bamya kök ağırlığını ise ortalama %14.5 oranında arttırdığı belirlenmiştir. Bitki yapraklarında makro-mikro besin içeriklerini ise kök-ur nematod uygulamasının kontrol bitkilere göre N, P, K ve Mg değerlerini ortalama olarak sırasıyla %2.31, 5.85, 5.24 ve 10.3 oranında azalttığı belirlenmiştir. Buna karşılık kök-ur nematod uygulamasının kontrol bitkilere göre Ca, Fe, Zn, Mn ve Cu değerlerini ortalama olarak sırasıyla %7.91, 17.0, 13.9, 118 ve 15.6 oranında arttırdığı belirlenmiştir.

Anahtar sözcükler: *Meloidogyne incognita*, morfolojik özellikler, besin elementi, bamya, dayanıklılık

[1] Alata Horticultural Research Institute, 33740, Erdemli, Mersin, Turkey
[2] Mersin University, Applied Technology and Management School of Silifke, 33940, Mersin, Turkey
* Corresponding author (Sorumlu yazar) e-mail: cetinnacar@hotmail.com
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Introduction

Okra [*Abelmoschus esculentus* (L.) Moench], a member of the Malvaceae, is commonly grown in tropical and subtropical regions of the world (Marin et al., 2017). Geographical origin of okra is thought to be Ethiopia and was initially distributed from there to southern Africa, the Mediterranean Basin, the Arabian Peninsula and South Asia, then the entire world (Sathish & Eswar, 2013).

Okra is largely consumed as human food and fruits are rich in vitamins, protein and crude fiber. With its carbohydrate, protein, oil, mineral and vitamin supply, okra is an important food for human nutrition (Abd El-Kader et al., 2010). Okra seeds are used as an oil source and fruits are also used as a fiber source. Carbohydrate and pectin-containing mucilage is obtained from the fruits and used as a thickener by the food industry (Alegbejo et al., 2008). Worldwide, almost 10 Mt of okra is produced on about 2.7 Mha (FAO, 2019). Okra is cultivated in all regions of Turkey. Commercial cultivation is common in Aegean, Marmara, Mediterranean and Central Anatolia Regions. In Turkey, annually 31 kt of okra is produced on 6 kha (FAO, 2019).

Stress is defined as conditions that influences or hampers plant growth, development and metabolism. Stress factors can be biotic or abiotic. Root-knot nematodes cause plant biotic stress resulting in serious economic losses in various plant species worldwide. Among the pests and diseases affecting okra production and limiting production area, root-knot nematode (*Meloidogyne* spp.*)* is the most important (Hussain et al., 2011). The symptoms of nematode infestation are slow growth rate, limited root development, leaf yellowing and wilting. Root-knot nematode causes wilting, chlorosis, stunted growth, and root galling, and when the nematode population exceeds the economic threshold level, it generally results in destruction of the roots, weak growth and reduced yield (Daramola et al., 2015; Mukhtar et al., 2017). Root-knot nematode-induced yield losses of 29 to 91% have been reported (Pandey & Kalra, 2003). Mukhtar et al. (2013) indicated *Meloidogyne incognita* (Kofoid & White, 1919) (Nematoda: Meloidogynidae) as the dominant species largely influencing plant growth and yield. Sikora and Fernandez (2005) reported that *Meloidogyne* spp. caused yield loss of up to 27% in okra. Subsequent cultivation of okra in the same field allows the nematodes to become widespread and colonizing the whole field (Hussain et al., 2011). In addition to these direct impacts, nematodes also increase *Fusarium* spp. infection, resulting greater damage and loss. In Turkey, *M. incognita*, *Meloidogyne arenaria* (Neal, 1889) and *Meloidogyne javanica* (Treub, 1885) (Nematoda: Meloidogynidae) have been reported as the most common and economically-damaging species in vegetable production (Kaşkavalcı & Öncüer, 1999; Özarslandan & Elekcioğlu, 2010; Çetintaş & Çakmak, 2016). Also, *Meloidogyne luci* can be a serious problem in field crops and greenhouse vegetables in northern areas of Turkey (Aydınlı, 2018).

For integrated management of nematodes, non-host plants are incorporated into the crop rotations, resistant cultivars are used, biological control and solarization are practiced, and nematicide and fumigants are applied. In large fields, due to the high cost, nematicides are not applied to okra. Generally non-host plants are incorporated into crop rotations in such contexts. Cultivars with nematode resistance usually give, greater yield than susceptible cultivars.

This study was conducted to determine the resistance of local okra cultivars against root-knot nematodes. Use of resistant cultivars in okra farming is the most effective, economic and practical method for control of nematodes. In this study, resistance of 26 okra cultivars against *M. incognita* was determined and nematode-induced morphological changes and effects on leaf macro- and micronutrients were determined.

Materials and Methods

Twenty-six okra from various geographic origins for Turkey were obtained from the large collection maintained at the Aegean Agricultural Research Institute, Menemen, Izmir, Turkey (Karagül, 2003). Twenty-four local okra cultivars, commonly cultivated in various regions of Turkey, and two commercially available and common local cultivars cv. Sultani (Marmara and Aegean Regions) and cv. Bornova (Aegean Region) were used in this study (Table 1). Testing of okra cultivars against nematodes were conducted in climate-controlled glasshouse at Alata Horticultural Research Institute in September-November 2019.

Resistance of the okra cultivars against root-knot nematode of *M. incognita* was investigated at 25 ± 1ºC and 60 ± 10% RH under controlled conditions. The root-knot nematode (*M. incognita*) used was produced on susceptible sivri pepper cv Alata F_1 (Alata Horticultural Research Institute, Mersin). At the end of nematode production period, egg masses on the roots were collected under a binocular microscope, and 2nd stage juveniles (J2s) were obtained with the aid of modified Baermann-funnel technique. J2s were counted under light microscope to prepare inoculum. Okra seeds were directly sown into pots. Experiments were conducted in a completely randomized design with four replicates. Treatments included a uninoculated control and nematode inoculation. The potting mix consisted of 80% sand, 5% silt and 15% soil disinfested by autoclaving. Each pot was inoculated with ~3,000 J2s at about 2 cm deep near plants that had grown to ~15 cm at the 2-4 leaf stage. Sixty days after inoculation (DAI), the plants were removed from the pots and root galls scored and morphological measurements made. Leaf samples were taken from the control and inoculated plants in each replicate and leaf macro- and micronutrients were determined. Nitrogen concentrations were determined in ground samples by a modified Kjeldahl method (Kacar, 1972). Phosphorus concentration were determined from the extracts obtained through wet digestion in nitric-perchloric acid mixture with the use of vanadomolybdophosphoric yellow method (Kacar & Kovancı, 1982). Potassium, calcium and magnesium, iron, zinc, manganese and copper concentrations were determined from the samen extracts using inductively coupled plasma (ICP) (Kacar, 1972).

Root gall index

Following the inoculation, plants were removed from the pots on 60 DAI and root galls were scored according to Hartman & Sasser (1985) based on 0-5 scale for number of eggs and galls. According to this scale, 0-2 indicates resistant and 3-5 indicates susceptible plants.

Plant measurements

Plant samples were arbitrary selected from each control (uninoculated) and nematode-inoculated pots 60 DAI and plant height (cm), root length (cm), leaf length (cm), leaf width (cm), fresh root weight (g) and stem thickness (mm) were measured.

Leaf macro- and micronutrients

Sixty DAI, leaf samples were taken from the control (nematode non-inoculated) and inoculated plants of each replicate and leaf macro- and micronutrients were determined. For this purpose, N, P, K, Ca and Mg as %, and Fe, Zn, Mn and Cu as ppm concentrations were determined.

Statistical Analysis

The relative effect of nematode inoculation on plant measurements and leaf macro- and micronutrients was determined with the use of Abbott formula [change = (1 − nematode-inoculated / control) × 100]. Experimental data were subjected to analysis of variance using of statistical analysis software (JMP v8.0.2.) and significant means were compared by Tukey HSD test at P = 0.05 significance level.

Results

Root gall index

Differences in root gall indexes were significant. Twenty-six okra cultivars were found to be nematode susceptible. Root gall indexes varied between 3-5 and an average value of 4.6 (Table 1). Differences between gall indexes of the cultivars were mainly attributed genetic characteristics of the plants. The greatest gall index (5) was obtained for cultivars 17, 19, 32, 40, 45, 57, 60, 62, 64, 68, 87, 88, 100 and 109 and the lowest (3.25) for cultivar 104 (Table 1). All local cultivars tested in present study were found to be susceptible to root-knot nematode (Figure 1).

Table 1. Local okra cultivars used in present study and gall indexes of 26 local okra cultivars

Cultivar No	Cultivar name	Origin	Index
3	TR 37126	Kastamonu	4.5
5	TR 39467	Kırklareli	4.8
11	TR 40293	Kırşehir	4.0
17	TR 42994	Konya	5.0
19	TR 43092	Kütahya	5.0
30	TR 57340	Sinop	4.0
32	TR 57344	Sinop	5.0
40	TR 61765	Trabzon	5.0
45	TR 69231	Karaman	5.0
56	TR 66081	Yozgat	4.5
57	TR 66070	Yozgat	5.0
60	TR 66087	Yozgat	5.0
62	TR 66076	Yozgat	5.0
64	TR 43185	Kütahya	5.0
67	TR 66581	Yozgat	4.0
68	TR 66594	Yozgat	5.0
84	Kılıçlı IIII	Mersin	3.8
87	Karşıyaka III	İzmir	5.0
88	Sultani Bamya	Marmara-Aegean Regions	5.0
89	Bornova Bamyası	Aegean Region	3.5
97	Tarsus Yalamık 1	Mersin	4.3
100	Şanlıurfa 1 Karaali	Şanlıurfa	5.0
104	Şanlıurfa 2	Şanlıurfa	3.2
108	Hilvan 3 Arpalı Köyü	Şanlıurfa	4.0
109	Siverek	Şanlıurfa	5.0
112	Denizli	Denizli	4.0
Mean			4.6

Figure 1. Nematode infestation response of three okra cultivars (17, 60 and 68).

Plant height, stem thickness and root length

In root-knot nematode-inoculated plants, the tallest plant height (35.8 cm) was in cultivar 45 and the shortest (16.8 cm) in cultivar 5. In control plants, the tallest plant height (67.5 cm) was in cultivar 87 and the shortest (34.3 cm) in cultivar 100. The change in plant height ranged from 17.3 to 71.3% with a mean of 46.0% (Table 2).

Table 2. Effects of root-knot nematode inoculation on plant growth parameters of 26 local okra cultivars

Cultivar No	Plant height (cm)			Stem thickness (mm)			Root length (cm)		
	Control*	Nematode	Change (%)	Control	Nematode	Change (%)	Control	Nematode	Change (%)
3	42.2 eh	21.7 hk	48.7	2.45 ij	3.29 dh	-34.3	19.7 ad	9.1 cf	53.7
5	54.6 be	16.8 k	69.2	5.39 a	3.24 di	39.9	17.7 bg	7.4 df	58.3
11	37.7 fh	28.4 be	24.6	3.05 gj	2.47 hi	19.0	18.6 af	8.9 cf	51.9
17	47.9 dg	23.7 ej	50.5	4.70 aj	3.67 cf	21.9	11.4 j	6.8 ef	40.4
19	49.7 df	23.2 fk	53.5	3.54 eh	3.22 di	9.0	15.8 ei	8.3 df	47.5
30	36.9 gh	29.9 bc	19.2	3.50 fi	5.49 a	-56.9	17.6 bg	8.4 df	52.6
32	54.9 ae	23.2 fk	57.8	5.04 ab	3.22 di	36.1	16.1 di	7.0 df	56.7
40	57.2 ad	31.6 ab	44.7	5.09 ab	3.04 ei	40.3	12.9 hj	10.2 be	20.8
45	64.5 ac	35.8 a	44.4	4.55 ae	3.82 ce	16.0	20.4 ab	13.0 ab	36.4
56	40.7 fh	20.3 ik	50.1	4.41 af	2.67 gi	39.5	15.3 fi	8.8 cf	42.6
57	48.0 dg	22.1 gk	54.0	4.62 aj	3.82 ce	17.3	22.2 a	15.5 a	30.3
60	48.9 dg	19.5 jk	60.3	2.89 gj	4.99 ab	-72.7	20.1 ac	15.1 a	25.1
62	32.8 h	20.6 ik	37.2	3.16 gj	3.64 cg	-15.2	14.7 gj	7.5 df	49.1
64	36.9 gh	26.8 bg	27.6	3.56 dh	3.85 ce	-8.2	14.5 gj	14.9 a	-3.3
67	36.7 gh	26.6 ch	27.6	4.25 bf	3.83 ce	9.9	20.0 ac	7.9 df	60.8
68	67.3 ab	19.3 jk	71.3	4.99 ab	3.07 ei	38.5	16.0 ei	7.8 df	51.0
84	36.5 gh	26.3 ch	27.8	2.72 gj	2.92 ei	-7.4	19.9 ac	9.2 cf	53.8
87	67.5 a	23.9 ej	64.6	4.61 ad	3.52 cg	23.6	15.1 fj	9.1 cf	39.3
88	33.1 h	27.4 bf	17.3	3.73 cg	3.65 cf	2.1	17.3 bg	14.4 a	17.0
89	37.7 fh	24.8 ci	34.1	5.09 ab	3.50 dg	56.7	15.3 fi	7.0 df	54.5
97	44.9 dh	20.7 ik	53.9	4.70 ac	2.75 fi	41.5	17.6 bg	5.7 f	67.7
100	34.3 h	26.0 ch	24.2	2.25 j	4.14 bd	-84.0	12.5 ij	10.5 bd	16.4
104	66.0 ac	29.2 bd	55.8	4.41 af	2.71 fi	38.6	19.3 ae	9.0 cf	53.6
108	66.3 ac	21.6 hk	67.5	2.63 hj	2.32 i	11.8	18.7 af	12.2 ac	35.0
109	54.1 ce	23.7 ej	56.1	5.43 a	3.45 dg	36.5	13.5 hj	13.4 ab	0.7
112	54.0 ce	24.6 di	54.4	5.32 a	4.48 bc	15.8	16.5 ch	13.2 ab	20.5
Mean	48.1	24.5	46.0	4.20	3.49	9.1	16.9	10.0	39.7
LSD (5%)	12.76	5.04		1.05	0.97		3.71	3.59	
F prob.	<0.001	<0.001		<0.001	<0.001		<0.001	<0.001	

* Control, uninoculated; nematode, nematode-inoculated.

In nematode-inoculated plants, the greatest stem thickness (5.49 mm) was in cultivar 30 and the lowest (2.32 mm) in cultivar 108. In control plants, the greatest stem thickness (5.43 mm) was in cultivar 109 and the lowest (2.25 mm) in cultivar 100. The change stem thicknesses ranged from -84.0% to 71.3% with mean of 9.06% (Table 2).

In nematode-inoculated plants, the greatest root length (15.1 cm) was in cultivar 57 and the lowest (5.70 cm) in cultivar 97. In control plants, the longest root length (22.2 cm) was in cultivar 57 and the shortest (11.4 cm) in cultivar 17. The change in root lengths ranged -3.3 to 67.7% with a of 39.7% (Table 2).

Root weight, leaf width and leaf length

In nematode-inoculated plants, the greatest root weight (5.77 g) was in cultivar 60 and the lowest (0.68 g) in cultivar 108. In control plants, the greatest root weight (2.56 g) was in cultivar 109 and the lowest (1.27 g) in cultivar 17. Root-knot nematode changed root weight by -258 to 60.2% with a mean of -14.5% (Table 3).

Table 3. Effects of root-knot nematode inoculation on plant growth parameters of 26 local okra cultivars

Cultivar No	Root weight (g)			Leaf width (cm)			Leaf length (cm)		
	Control*	Nematode	Change (%)	Control	Nematode	Change (%)	Control	Nematode	Change (%)
3	1.84 dg	1.68 df	8.7	8.4 gi	10.6 bc	-26.9	7.8 hi	9.0 cd	-15.5
5	2.44 ab	0.98 gk	59.8	14.3 ce	6.6 h	54.0	11.4 ad	6.3 h	44.5
11	1.68 eh	1.26 fk	25.0	7.6 i	12.0 ab	-57.2	9.0 fi	7.4 dh	17.8
17	1.27 eh	4.01 b	-215.8	8.2 hi	8.7 cg	-6.4	8.1 hi	7.9 dh	3.1
19	1.47 eh	1.52 ei	-3.4	11.9 ae	9.3 ce	21.5	11.0 ae	8.5 cf	22.7
30	2.40 ac	1.47 ei	38.8	11.5 be	9.2 cf	20.6	12.0 ac	8.6 ce	28.2
32	1.34 fh	1.27 fk	5.2	11.1 cf	7.5 eh	32.4	11.0 ae	6.6 gh	39.5
40	1.29 gh	2.40 c	-86.1	13.3 ac	9.5 ce	29.1	12.1 ac	9.1 cd	25.0
45	1.86 cf	2.15 ce	-15.6	11.6 be	9.1 cg	21.7	11.0 ae	8.6 ce	22.5
56	1.77 dh	1.31 fk	26.0	12.0 ae	7.7 dh	36.2	11.6 ad	7.5 dh	35.7
57	1.43 eh	1.36 fk	4.9	10.6 dg	8.1 dh	23.6	10.7 bf	7.6 dh	28.4
60	1.61 eh	5.77 a	-258.4	13.0 ad	12.7 a	2.3	11.2 ad	10.9 ab	2.9
62	1.50 eh	0.70 jk	53.3	9.0 fi	7.2 gh	20.1	9.3 eh	7.5 dh	20.2
64	1.41 eh	1.86 cf	-31.9	10.4 eh	7.3 fh	30.5	10.1 dg	6.9 eh	32.1
67	1.68 eh	1.57 dh	6.6	12.1 ae	8.3 dh	31.5	10.8 bf	7.7 dh	28.6
68	1.61 eh	0.85 ik	47.2	11.1 cf	11.5 ab	-3.6	11.6 ad	11.1 ab	4.1
84	1.55 eh	0.88 hk	43.2	12.1 ae	9.1 cf	24.4	10.9 be	8.4 cf	23.0
87	1.31 fh	2.45 c	-87.0	10.6 dg	9.5 cd	10.1	10.4 cg	8.9 cd	14.4
88	1.42 eh	1.80 cf	-26.8	11.9 ae	9.3 ce	22.1	10.8 bf	8.2 cg	24.2
89	1.52 eh	1.66 dg	-9.2	13.1 ac	9.5 cd	27.1	11.2 ad	8.2 cg	27.1
97	2.25 ad	1.34 fk	40.4	8.1 hi	7.5 eh	7.4	7.8 i	6.9 eh	11.5
100	1.68 eh	2.24 c	-33.3	8.7 gi	10.4 bc	-19.2	8.7 gi	9.7 bc	-11.5
104	1.49 eh	1.39 fj	6.7	13.1 ac	8.1 dh	38.3	11.2 ad	6.8 fh	39.3
108	1.71 dh	0.68 k	60.2	13.9 ab	9.0 cg	35.8	12.7 a	7.9 dh	38.2
109	2.56 a	3.66 b	-43.0	14.4 a	9.2 cf	35.9	12.4 ab	8.3 cg	33.2
112	1.94 be	1.81 cf	6.7	11.3 cf	13.4 a	-18.9	11.6 ad	12.2 a	-5.9
Mean	1.69	1.85	-14.5	11.3	9.23	15.1	10.6	8.32	20.5
LSD (5%)	0.56	0.70		2.44	1.97		1.82	1.74	
F prob.	<0.001	<0.001		<0.001	<0.001		<0.001	<0.001	

* Control, uninoculated; nematode, nematode-inoculated.

In nematode-inoculated plants, the greatest leaf width (13.4 cm) was in cultivar 112 and the lowest (6.58 cm) in cultivar 5. In control plants, the greatest leaf width (14.4 cm) was in cultivar 109 and the lowest (7.60 cm) in cultivar 11. The change in leaf width ranged from -57.2 to 54.0% with a mean of 15.1% (Table 3).

In nematode-inoculated plants, the greatest leaf length (12.2 cm) was in cultivar 112 and the lowest (6.30 cm) in cultivar 5. In control plants, the greatest leaf length (12.7 cm) was in cultivar 108 and the lowest (7.50 cm) in cultivar 97. The change in leaf length ranged from -15.5 to 44.5% with a mean of 20.5% (Table 3).

Nitrogen, phosphorus and potassium

In nematode-inoculated plants, the greatest N concentration (2.39%) was in cultivar 100 and the lowest (1.19%) in cultivar 17. In control plants, the greatest N concentration (2.76%) was in cultivar 109 and the lowest (1.28%) in cultivar 19. The change ranged from 47.1 to 73.4% with a mean of 2.31% (Table 4).

In nematode-inoculated plants, the greatest P concentration (0.35%) was in cultivar 11 and the lowest (0.13%) in cultivar 62. In control plants, the greatest P concentration (0.55%) was in cultivar 109 and the lowest (0.17%) in cultivar 19. The change ranged from -50% to 67% with a mean of 5.85% (Table 4).

In nematode-inoculated plants, the greatest K concentration (1.80%) was in cultivar 100 and the lowest (0.55%) in cultivar 62. In control plants, the greatest K concentration (2.43%) was in cultivar 57 and the lowest (0.60%) in cultivar 62. The change ranged from -86.1% to 63.8% with a mean of 5.24% (Table 4).

Table 4. Nematode-induced changes in leaf N, P and K concentrations of 26 local okra cultivars

Cultivar No	N (%)			P (%)			K (%)		
	Control*	Nematode	Change (%)	Control	Nematode	Change (%)	Control	Nematode	Change (%)
3	2.03 gi	1.37 j	32.5	0.18 lm	0.14 jk	22.2	1.24 kj	0.74 m	40.3
5	1.95 ij	2.24 bc	-14.9	0.19 km	0.21 df	-10.5	0.86 n	1.60 b	-86.1
11	2.11 fh	2.36 ab	-11.9	0.28 bd	0.36 a	-28.6	0.92 mn	1.17 fj	-27.2
17	2.17 eg	1.19 k	45.2	0.27 be	0.16 jk	40.7	1.39 hi	0.69 m	50.4
19	1.28 m	2.22 bd	-73.4	0.17 m	0.23 ce	-35.3	0.99 m	1.62 b	-63.6
30	1.75 k	2.33 ab	-33.1	0.20 im	0.30 b	-50.0	0.88 n	1.40 cd	-59.1
32	2.01 hi	1.56 i	22.4	0.26 dg	0.21 df	19.2	2.28 b	1.14 gj	50.0
40	2.40 b	1.39 j	42.1	0.28 bd	0.16 hk	42.9	1.52 fg	1.09 ik	28.3
45	2.29 be	2.04 e	10.9	0.24 ei	0.19 fh	20.8	1.31 ij	1.02 k	22.1
56	1.82 jk	2.27 ac	-24.7	0.20 im	0.24 cd	-20.0	1.23 jl	1.40 cd	-13.8
57	2.36 bc	1.35 j	42.8	0.30 bc	0.17 hj	43.3	2.43 a	0.88 l	63.8
60	2.23 cf	1.72 gh	22.9	0.22 hl	0.17 gj	22.7	1.14 kl	1.32 de	-15.8
62	2.38 b	1.26 jk	47.1	0.27 cf	0.13 k	51.9	1.48 gh	0.55 n	62.8
64	1.53 i	2.22 bd	-45.1	0.19 km	0.23 ce	-21.1	0.87 e	1.20 fh	-37.9
67	2.29 be	1.39 j	39.3	0.22 gk	0.20 eg	9.1	1.41 gi	1.08 jk	23.4
68	2.26 be	1.25 jk	44.7	0.21 im	0.17 hj	19.1	1.62 ef	0.66 m	59.3
84	1.906 ik	2.27 ac	-19.5	0.22 gk	0.25 c	-13.6	1.13 l	1.27 ef	-12.4
87	2.03 gi	2.34 ab	-15.3	0.21 im	0.25 c	-19.1	1.49 gh	1.25 eg	16.1
88	1.32 m	2.15 ce	-62.9	0.31 b	0.23 ce	25.8	1.87 c	1.39 cd	25.7
89	2.19 df	2.36 ab	-7.8	0.25 dh	0.24 cd	4.0	1.34 ij	1.20 fi	10.5
97	2.32 bd	2.07 de	10.8	0.23 fj	0.23 ce	0.0	1.18 kl	1.16 gj	1.7
100	2.22 cf	2.39 a	-7.7	0.22 gk	0.26 c	-18.2	1.50 g	1.80 a	-20.0
104	1.94 ij	1.87 f	3.6	0.21 im	0.21 df	0.0	1.72 de	1.11 hk	35.5
108	1.75 k	1.83 fg	-4.6	0.20 jm	0.21 df	-5.0	0.60 o	1.02 k	-70.0
109	2.76 a	1.63 hi	40.9	0.56 a	0.19 fi	66.1	1.80 cd	0.87 l	51.7
112	1.89 ik	2.35 ab	-24.3	0.21 im	0.24 cd	-14.3	1.69 e	1.68 b	0.6
Mean	2.05	1.90	2.3	0.24	0.21	5.9	1.38	1.17	5.2
LSD (5%)	0.15	0.15		0.04	0.03		0.11	1.11	
F prob.	<0.001	<0.001		<0.001	<0.001		<0.001	<0.001	

* Control, uninoculated; nematode, nematode-inoculated.

Calcium, magnesium and iron

In nematode-inoculated plants, the greatest Ca concentration (4.70%) was in cultivar 100 and the lowest (2.91%) in cultivar 56. In control plants, the greatest Ca concentration (4.84%) was in cultivar 87 and the lowest (2.73%) in cultivar 64. The change ranged from -59.0% and 24.4% with a mean of -7.9%. However, nematode inoculation generally increased Ca concentrations (Table 5).

In nematode-inoculated plants, the greatest Mg concentration (0.89%) was observed in cultivar 68 and the lowest (0.53%) in cultivar 100. In control plants, the greatest Mg concentration (0.98%) was obtained from cultivar 17 and the lowest (0.52%) from cultivar 64. The change ranged from 17.7 to -38.8% with a mean of 10.3%. However, nematode inoculation generally reduced Mg concentrations (Table 5).

In nematode-inoculated plants, the greatest Fe concentration (289 ppm) was in cultivar 100 and the lowest (76 ppm) in cultivar 19. In control plants, the greatest Fe concentration (331 ppm) was in cultivar 57 and the lowest (85 ppm) in cultivar 30. The change ranged from -155% and 56.0% and with a mean of -17.0%. However, nematode inoculation increased Fe concentrations (Table 5).

Table 5. Nematode-induced changes in leaf Ca, Mg and Fe concentrations of 26 local okra cultivars

Cultivar No	Ca (%)			Mg (%)			Fe (ppm)		
	Control*	Nematode	Change (%)	Control	Nematode	Change (%)	Control	Nematode	Change (%)
3	3.39 il	3.98 ce	-17.4	0.68 j	0.66 eh	2.9	130 f	82 mn	37.5
5	3.69 ei	3.44 i	6.8	0.85 d	0.70 dg	17.7	105 im	111 1jk	-5.4
11	3.41 4ik	3.87 df	-13.5	0.84 de	0.71 df	15.5	91 no	110 jk	-21.0
17	4.15 2bc	4.37 b	-5.3	0.98 a	0.60 hj	38.8	165 d	110 jk	33.2
19	3.22 km	3.49 hi	-8.4	0.80 eg	0.87 ab	-8.8	92 mo	76 n	17.2
30	3.49 hk	3.53 hi	-1.2	0.90 bc	0.61 gj	32.2	85 o	105 kl	-23.4
32	3.90 cf	3.79 fg	2.8	0.74 hi	0.65 eh	12.2	291 b	128 hi	56.0
40	3.84 dg	4.03 cd	-5.0	0.73 i	0.63 fi	13.7	111 gk	228 b	-105.1
45	3.60 gj	3.84 ef	-6.7	0.76 gi	0.64 fi	15.8	104 in	137 gh	-31.9
56	3.58 gj	2.91 j	18.7	0.78 fh	0.70 dg	10.3	107 hl	186 cd	-73.0
57	4.02 bd	4.09 c	-1.7	0.84 de	0.71 df	15.5	331 a	180 de	45.6
60	3.97 ce	3.42 i	13.9	0.86 cd	0.63 fi	26.7	91 no	83 mn	8.6
62	4.20 bc	4.35 b	-3.6	0.86 cd	0.71 df	17.4	112 gj	105 kl	6.1
64	4.84 a	3.66 gh	24.4	0.52 l	0.55 ij	-5.8	121 fg	114 jk	6.1
67	3.58 gj	4.44 b	-24.0	0.82 df	0.77 cd	6.1	94 lo	172 ef	-81.8
68	3.31 jm	4.37 b	-32.0	0.82 df	0.89 a	-8.5	113 gi	127 hi	-13.1
84	3.42 ik	4.04 cd	-18.1	0.75 hi	0.67 eh	10.7	97 ko	198 c	-103.1
87	2.73 n	4.34 b	-59.0	0.62 k	0.73 de	-17.7	98 jo	138 gh	-41.2
88	3.77 dh	3.54 hi	6.1	0.77 gi	0.65 eh	15.6	173 cd	120 ij	30.3
89	3.01 mn	2.94 j	2.3	0.84 de	0.67 eh	20.2	107 il	108 jl	-0.8
97	3.62 fi	4.02 ce	-11.1	0.76 hi	0.83 ac	-9.2	96 lo	144 g	-50.9
100	4.28 b	4.70 a	-9.8	0.63 k	0.53 j	15.9	114 gi	289 a	-154.7
104	3.97 be	4.01 ce	-1.0	0.73 i	0.78 bd	-6.9	186 c	129 hi	31.0
108	3.69 ei	4.28 b	-16.0	0.91 b	0.78 bd	14.3	121 fh	121 ij	-0.2
109	3.29 jm	4.27 b	-29.8	0.76 gi	0.64 ei	15.8	151 e	162 f	-7.1
112	3.09 lm	3.62 gh	-17.2	0.68 j	0.63 fi	7.4	94 lo	95 lm	-0.6
Mean	3.66	3.90	-7.9	0.78	0.69	10.3	130	137	-17.0
LSD (5%)	0.30	0.18		0.05	0.09		13.8	13.6	
F prob.	<0.001	<0.001		<0.001	<0.001		<0.001	<0.001	

* Control, uninoculated; nematode, nematode-inoculated.

Zinc, manganese and copper

In nematode-inoculated plants, the greatest Zn concentration (113 ppm) was in cultivar 109 and the lowest (12.3 ppm) in cultivar 68. In control plants, the greatest Zn concentration (392 ppm) was in cultivar 57 and the lowest (12.5 ppm) in cultivar 19. The change ranged from -212% to 94.4% and mean increase in Zn concentrations was calculated as -13.9% (Table 6). However, nematode inoculation generally increased Zn concentrations

In nematode-inoculated plants, the greatest Mn concentration (336 ppm) was in cultivar 30 and the lowest (41.2 ppm) in cultivar 3. In control plants, the greatest Mn concentration (98.0 ppm) was in cultivar 89 and the lowest (32.4 ppm) in cultivar 108. The change ranged from -744% to 24.8% with a mean of -118% (Table 6). However, nematode inoculation generally increased Mn concentrations.

In nematode-inoculated plants, the greatest Cu concentration (6.59 ppm) was in cultivar 100 and the lowest (1.79 ppm) in cultivar 57. In control plants, the greatest Cu concentration (8.89 ppm) was in cultivar 57 and the lowest (1.34 ppm) in cultivar 60. The change ranged from -129% to 79.9% and mean increase in Cu concentrations was calculated as -15.6% (Table 6). However, nematode inoculation generally increased Cu concentrations.

Table 6. Nematode-induced changes in leaf Zn, Mn and Cu concentrations of 26 local okra cultivars

Cultivar No	Zn (ppm)			Mn (ppm)			Cu (ppm)		
	Control*	Nematode	Change (%)	Control	Nematode	Change (%)	Control	Nematode	Change (%)
3	132.3 c	15.0 ln	88.7	43.7 jl	41.2 o	5.8	2.15 p	3.90 f	-81.4
5	23.5 fg	29.1 h	-23.4	36.0 mn	75.5 lm	-110.0	2.09 p	4.01 f	-91.9
11	17.9 gi	23.8 ij	-32.7	38.0 ln	54.9 no	-44.6	3.38 ik	2.95 ij	12.7
17	128.5 c	13.4 mn	89.6	71.2 de	141.4 ef	-98.6	5.04 e	2.78 j	44.8
19	12.5 i	14.9 ln	-19.2	60.7 gh	45.7 o	24.8	3.26 jl	3.51 gh	-7.7
30	13.2 hi	41.2 e	-212.1	39.9 lm	336.4 a	-743.9	1.96 p	3.29 h	-67.9
32	329.4 b	18.5 kl	94.4	79.9 c	64.6 mn	19.2	6.98 b	5.22 c	25.2
40	30.5 f	39.1 ef	-28.2	64.3 fg	116.8 gh	-81.6	4.28 g	4.39 e	-2.6
45	25.8 fg	23.6 ij	8.6	68.4 ef	205.1 c	-199.9	4.70 f	3.97 f	15.5
56	23.8 fi	33.8 g	-42.4	54.5 i	48.8 no	10.5	2.96 mn	4.09 ef	-38.2
57	329.4 a	51.6 d	84.3	92.6 ab	96.4 ik	-4.0	8.89 a	1.79 m	79.9
60	27.1 fg	35.3 fg	-30.2	41.2 km	119.0 g	-189.1	1.34 q	2.34 k	-74.6
62	20.9 fi	14.0 mn	32.9	80.4 c	80.1 km	0.4	2.66 o	3.28 hi	-23.3
64	20.7 fi	60.2 c	-190.9	89.1 b	220.4 c	-147.3	2.54 o	5.81 b	-128.7
67	17.4 gi	12.9 mn	26.1	54.8 hi	109.4 gi	-99.7	2.78 no	2.18 kl	21.6
68	24.2 fh	12.3 n	49.3	56.6 hi	55.9 no	1.2	2.96 mn	3.92 f	-32.4
84	16.1 gi	26.6 hi	-65.3	57.7 hi	174.1 d	-201.7	3.50 ij	5.28 c	-50.9
87	20.6 fi	19.9 jk	3.5	54.9 hi	100.0 hj	-82.2	2.71 no	3.80 fg	-40.2
88	104.9 d	47.2 d	55.0	48.1 j	262.0 b	-444.7	6.52 c	4.88 d	25.2
89	21.4 fi	19.9 jk	7.1	98.0 a	150.4 e	-53.5	3.61 i	3.35 h	7.2
97	24.3 fh	65.5 b	-169.1	47.0 jk	98.2 ik	-109.1	2.64 o	4.00 f	-51.5
100	15.5 gi	35.3 fg	-127.9	66.3 eg	176.7 d	-166.6	3.20 km	6.59 a	-105.9
104	78.9 e	16.8 kn	78.8	65.0 fg	88.4 jl	-36.1	4.76 f	2.27 k	52.3
108	21.7 fi	17.4 km	20.2	32.4 n	83.8 jl	-158.5	3.99 h	2.25 kl	43.6
109	70.1 e	112.8 a	-60.8	76.8 cd	127.4 fg	-66.0	3.12 lm	3.29 h	-5.5
112	19.5 fi	18.8 kl	3.4	81.8 c	158.3 de	-93.6	6.20 d	1.94 lm	68.7
Mean	60.4	31.5	-13.9	61.5	124.3	-118.0	3.78	3.66	-15.6
LSD (5%)	11.66	4.51		5.94	18.46		0.25	0.32	
F prob.	<0.001	<0.001		<0.001	<0.001		<0.001	<0.001	

* Control, uninoculated; nematode, nematode-inoculated.

Discussion

In Turkey, resistance of local okra cultivars against *M. incognita* has not been studied previously. Okra is severely damaged by root-knot nematodes and most cultivars are susceptible to *M. incognita*. Twenty-six cultivars were not resistant to *M. incognita.* Root gall indexes for the local okra cultivars varied between 3.3 and 5 with a mean of 4.6 indicating that these cultivars as susceptible to the nematode. Similar studies have been conducted elsewhere, especially in Asian and African countries. Hussain et al. (2014) conducted a study under field conditions in three regions of Pakistan with 12 okra cultivars and reported root gall indexes as between 4 and 5. Basil et al. (2019) inoculated 10 okra cultivars and reported root gall indexes as between 3.7 and 4.2. Karajeh & Salameh (2015) conducted a nematode inoculation study in Jordan with 36 okra cultivars and reported moderate resistance against the nematode. Kedarnath et al. (2017) conducted a study in India on seven okra cultivars and reported gall indexes of 3 to 4 for plants 60 DAI and at harvest. These results are similar to the present study, but not all their results. Sheela et al. (2006) investigated root gall indexes of 123 okra cultivars and based on gall index of 3 reported that theses okra cultivars were moderately resistant. Similarly, Muhammad et al. (2017) conducted a study under field conditions in Pakistan with 12 okra cultivars and reported root gall indexes of 2-4. As seen in previous studies, okra is generally not resistant to root-knot nematodes. Although a range of research has evaluated the reaction of okra cultivars to root-knot nematodes, there are no reports of fully resistant cultivars, only those that are moderately resistant and susceptible (Mohanta & Mohanty, 2012; Hussain et al., 2014; Marin et al., 2017).

No studies similar to the present study have been reported, but a few studies examined plant growth parameters in okra, tomatoes and soybeans. In present study, effects of *M. incognita* inoculation on plant growth parameters were investigated. Compared to uninoculated control plants, root-knot nematode inoculation reduced plant height, stem thickness, root length, leaf width and leaf length but root weight was increased depending on gall formation. Kumar et al. (2012) investigated the effects of nematode inoculation on root length, shoot length, fresh and dry root weights and reported significantly effects. Root and shoot lengths were greater in control plants. Mean root length of the control and inoculated plants was reported as 40 and 32 cm, shoot length as 49 and 32 cm, root dry weights as 4.8 and 7.0 g and root fresh weights as 13.4 and 11.5 g, respectively. Odeyemi et al. (2016) reported *M. incognita*-induced reductions of between 13.7 and 75.6% in plant height, between 13.7 and 67.2% plant fresh weight and between 13.0 and 53.3% in fruit weight. In another study conducted on okra, Claudius-Cole (2018) reported mean number of fruits as 18.4 for control plants and 13.8 for *M. incognita*-inoculated plants; mean fruit weight as 167 g in control plants and 81.3 g for inoculated plants. Yield loss was reported as 51.4%. Pandey et al. (2019) reported (60 DAI) significant effects of nematode inoculation on plant length, plant diameter, root length and root diameter and reported. Plant length, plant diameter, root length and root diameter were greater in control plants in current study. Azam et al. (2011) examined *M. luci* infestation in tomatoes and reported decreased plant height and weights with increasing Pi density. Root-knot nematode symptoms in aboveground plant parts are reported to be stunted growth, chlorosis in leaves and reductions in plant weight. Gall formation-induced browning in leaves and recessed plant growth are generally encountered in nematode-infested okras and yield reductions vary between 29 and 91% (Pandey & Kalra, 2003). Studies on soybean and cucumber found that root-knot nematodes infestation increased root weights (Kayani et al., 2017). There was a general reduction in the growth parameters measured for the three okra cultivars for infested versus uninfested plants. The present findings on plant growth parameters are consistent with the findings of earlier studies. As a result, the galls formed in response to the feeding the root-knot nematodes disrupt the structure of the root and prevent uptake of water and nutrients from the soil, changing the distribution of photosynthesis products in the plant, increasing the movement of these products towards the root region, especially during the development and reproduction of the nematode. As a result, the inhibition of plant growth causes a decrease in plant height and stem diameter, and shrinkage of leaves, shortening of plant root, increase in plant root diameter and increase in plant root weight (Fortnum et al., 1991; Carneiro et al., 1999; Maleita et al., 2012).

Plant-pathogen-nutrient interactions are complex and the mechanisms have not been fully elucidated. Nutrients are essential elements for plant growth and development, and are essential for various physiological processes including protein synthesis, photosynthetic electron transfer, mitochondrial respiration, oxidative stress responses, cell wall metabolism and hormonal structure (Dordas, 2008; Santana-Gomes et al., 2013). In the present study, the concentrations of macro- and micronutrients were investigated in the leaves of okra cultivars. Compared to uninoculated control plants, nematode inoculation reduced leaf N, P, K and Mg concentrations and increased Ca, Fe, Zn, Mn and Cu concentrations. While macronutrients are generally reduced by nematode infestation, micronutrient accumulation was higher in leaf tissues.

There has been no reported study on the nematode effect of the concentrations of macro- and micronutrients in plant leaves. Similar studies have been done with plants such as tomatoes, soybeans, ridge gourd and coffee. Therefore, comparisons are made here with these plants. Miamoto et al. (2017) conducted a study on soybean and reported greater leaf Ca, Fe, Zn, Mn and Cu concentrations in nematode, *Pratylenchus brachyurus* (Godfrey, 1929) (Nematoda: Pratylenchidae), inoculated plants than in control plants. S, Cu and Zn, cofactors of plant enzymes and influencing cell wall formation and composition, produce some substances and improve plant resistance against nematodes through generating a physical barrier. Dietrich et al. (2004) reported lower N, Ca, Mg, Fe and Mn concentrations in nematode-infested plants [*Arabidopsis thaliana* (L.) (Tracheophyta: Brassicaceae)] than in control plants. Activation of plant defense mechanism increased energy consumption and thus reduced the concentrations of these nutrients. In contrast, Carneiro et al. (2002) reported greater Mg, Fe, Mn and Zn concentrations in nematode-inoculated soybean plants. N and Ca concentrations were lower in shoots than in root-knot nematode-infested roots. Pathogens alters plant nutrient concentrations. Goncalves et al. (1995) indicated that *M. incognita* reduced P, Mg, Fe, Mn and B concentrations of coffee plants. Blevins et al. (1995) reported high Ca and low K and Mn concentrations in *Heterodera glycines* Ichinohe, 1952 (Nematoda: Heteroderidae) nematode-infested roots of soybean. Hajji et al. (2016) reported that nematodes significantly increased Cu, Zn, Fe, Mn, Mg and K concentrations and decreased Ca concentration in tomato roots. Carneiro et al. (2002) reported decreased N and P and increased Ca concentration in nematode-infested soybean roots. Decreased leaf size of nematode-infested plants resulted in increased Ca concentrations. Melakeberhan et al. (1985) reported that *M. incognita* reduced plant dry weight and increased Ca concentration of bean plants. Pandey et al. (2019) reported that as compared to the control plants, *M. incognita* reduced plant protein (20.4 vs 40.3%), N (20.0 vs 40.4%) and P (11.4 vs 92.0%) concentrations and increased K (27.3 vs 208%) concentration of ridge guard. Pandey et al. (2017) compared the control and *M. incognita*-infested mung bean plants and compared to the control plants, N concentrations of nematode-inoculated plants decreased by 4.1 to 88.6%, protein concentrations by 4.1 to 88.5%, P concentrations by 4.6 to 52.3% and K concentrations by 7.1 to 37.9%. Hurchanik et al. (2004) reported that nematode infestations significantly reduced Ca, Mg, P and B concentrations and increased Mn, Cu, Zn concentrations and Ca/B ratio in roots of coffee plants. Macro- and micronutrients present in okra are responsible for plant growth and development. According to the authors, nematode infestation causes abiotic and biotic stress in plants. As a result, nutrient uptake and accumulation of plants are affected. Root-knot nematode infestations cause physiological and biochemical changes. Such changes cause cell growth and tissue thickening in the roots, affecting the water nutrient uptake of the roots. As a result, it negatively affects plant growth and reduce yield. (Hussain et al., 2016; Débia et al., 2019).

Conclusion

In present study, root-knot nematode induced large and small galls on roots of local okra cultivars and all cultivars were identified as susceptible to the nematode. Similar findings were also reported for previous studies conducted in different parts of the world. Nematode damage impaired plant water and nutrient uptake from the soil, thus resulted in stunted growth. Compared to uninoculated control plants, nematode inoculation reduced plant height, stem thickness, root length, leaf width and leaf length, and increased root weight. Leaf samples were taken from the control and inoculated plants and leaf macro- and micronutrients were determined. It was observed that nematode inoculation reduced leaf N, P, K and Mg concentrations, but increased Ca, Fe, Zn, Mn and Cu concentrations. Since plant-pathogen-nutrient interactions are complex and the mechanisms involved have not been fully elucidated, further research is recommended on these mechanisms. Given environmental hazards and potential residues, nematicides are not recommended in okra production. Fumigation is hard to implement over large fields. Therefore, in control of root-knot nematode in okra fields, incorporation of non-host plants into crop rotations is recommended. Further breeding studies are recommended to identify the resistance gene of okras against root-knot nematode and to develop resistant cultivars.

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