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Response of some carnation varieties to *Meloidogyne incognita*, *Meloidogyne javanica* and *Meloidogyne arenaria*

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Abstract
Carnation is one of the most crucial cut flowers in Turkey. Root-knot nematodes (RKNs) cause significant damage on carnation production and quality. Therefore, determination of response of carnation varieties to RKNs is required for management practices. In this study, four carnation cultivars, Turbo, Betsy, Nirvana and Glaciar, were inoculated with second stage juveniles (J2s) of *Meloidogyne incognita*, *M. javanica* and *M. arenaria* under controlled conditions. *Meloidogyne incognita*, *M. javanica* and *M. arenaria* did not reproduce on Turbo variety. Therefore, Turbo variety was found to be resistant to these nematodes. Nirvana variety was moderately resistant to *M. incognita* and *M. javanica*, but highly resistant to *M. arenaria*. Betsy and Glaciar varieties were susceptible to *M. incognita*, *M. javanica* and *M. arenaria*. Results showed that the response of carnations was different based on root knot nematodes. Determining resistant carnation varieties could be an advantage for the control of RKNs. Resistant carnations could be used to control the RKNs and develop new carnation varieties.

Keywords
Carnation, Resistance, *Meloidogyne incognita*, *Meloidogyne javanica*, *Meloidogyne arenaria*

Introduction
Carnation (*Dianthus caryophyllus* L.) is one of the major cut flowers and has economic value in the world. It belongs to the genus *Dianthus* which is a member of the family Caryophyllaceae. More than 300 species have been reported (Galbally and Galbally, 1997; Jurgens et al., 2003). Cut flowers of carnation are classified into two types as standard and spray. The standard and spray types have one flower and multiple flowers on a stem, respectively (Satoh et al., 2005). Carnation, rose and chrysanthemum form more than 50% of cut flower market in the world. The main carnation importer countries in the European market are the United Kingdom, the Netherlands and Germany. The major suppliers of carnations to the EU are Colombia, Turkey, Kenya, Morocco, and Ethiopia, respectively (Anonymous, 2017). In Turkey, carnations are widely grown and produced cut flowers with 607.070.350 pieces. Also, Turkey is the 3rd country to export the largest amount of carnation with $32,4 million (Trademap, 2018; Turkish Statistical Institute, 2018).

Carnation production is affected by many diseases and pests (Trujillo et al., 1989; Marroquin and Arbelaez, 1992; Sharma and Sharma, 2008;). However, there are limited studies on interaction between carnation and nematode. RKNs are major pathogens in carnation areas (Lamberti et al., 1987; Çelik et al., 2019). Nagesh and Parvatha Reddy (2000) showed that *Meloidogyne incognita* caused 26% yield loss in the carnation production in India. In another study, Phyllis (1997)

reported that yield loss caused by RKNs was 20% worldwide.

Management of the RKNs is very difficult since they can survive in soil and plant tissues. Various cultural, physical, biological and chemical control methods have been used against *Meloidogyne* spp. (Thomason and Caswell, 1987). Nematicides application against root knot nematodes is commonly used but the use of highly toxic nematicides adversely affects human health and the environment (Stirling, 1991; Rich et al., 2004). Therefore, host plant resistance to root-knot nematodes provides a successful alternative management strategy. Fawzy et al. (1991) reported two carnation cultivars were resistant to *M. incognita* among twelve carnation cultivars tested. In another study, Cho et al. (1996) investigated thirty-three carnation cultivars to determine the resistance to *M. incognita* and determined that seven carnation cultivars were resistant. Since carnation production is the most significant cut flower sector in Turkey, a survey was carried out and reported that RKNs were present widespread in carnation production areas (Çelik et al., 2019). However, there is no study on resistance of carnation cultivars to root knot nematode in Turkey. Therefore, in this study, the response of some carnation cultivars that are widely grown in Turkey was investigated against *M. incognita*, *M. javanica* and *M. arenaria* isolates under controlled conditions.

Materials and Methods

Plant material

Carnation varieties used in this study are listed in Table 1. Cuttings obtained from each carnation cultivar were treated with humic acid for better rooting. The rooted cuttings were transplanted to 125 ml plastic pots containing sandy loam soil sterilized with an autoclave. The seedlings were watered daily for the duration of the experiment.

Table 1. The type and company of carnation varieties used in this study

Variety	Type	Company
Turbo	Standard	Santa Maria
Betsy	Standard	Selecta
Nirvana	Spray	Selecta
Glaciar	Spray	Dümmen Orange

Root-knot nematodes

Meloidogyne incognita, *M. javanica* and *M. arenaria* isolates were used in this study. They were identified in the previous study (Devran and Söğüt, 2009). The isolates have been cultured in our laboratory for a long time.

Nematode culture

The isolates were multiplied on susceptible tomato cv. Tueza F1. Tomato seedlings were inoculated with 1000 J2 according to Özalp and Devran (2018). Then, tomato plants were maintained under controlled conditions (25 ± 1 °C temperature, 16-h light/8-h dark photoperiod and 60 ± 5% relative humidity). Eight weeks later, plants were removed from soil. The egg masses were collected from roots of tomato infected using a needle and hatched at room temperature. After then J2s were counted under a light microscope They were stored in the refrigerator at 4ºC for 2 days until inoculation.

Nematode inoculation and experimental design

Carnation cultivars were separately inoculated with 500 *M. incognita*, *M. javanica* and *M. arenaria* J2. The studies were performed as a completely randomized block design with 5 replications and repeated twice. Carnation cultivars were incubated in the growth chamber under the conditions mentioned above and harvested 8 weeks after inoculation. The roots of plants were washed free of soil individually under tap water. Each root system was stained with phloxine B and assessed based on 0-10 Zeck-scale (0=no galls, 1=very few small galls, 2=numerous small galls, 3=numerous small galls of which some are grown together, 4=numerous small and some big galls, 5=25% of roots severely galled, 6=50% of roots severely galled, 7=75% of roots severely galled, 8=no healthy roots but plant is still green, 9=roots rotting and plant dying, 10=plant and roots dead) (Zeck, 1971).

Statistical analyses

Data on nematode infestation scores were analyzed by ANOVA. The significance of the differences among mean values was tested with Tukey's test at the $P \leq 0.05$ significance level. The analysis was carried out with SAS (v. 9.0 for Windows; SAS Institute Inc., Cary, NC, USA).

Results and Discussion

Production of quality carnation against RKNs is quite important for commercial flower growers. In this study, the response of four carnation varieties grown widely in Turkey was investigated to isolates of *M. incognita*, *M. javanica* and *M. arenaria*. Reactions of Turbo and Betsy, which are cultivars of standard type, were found to be significantly different to *M. incognita*, *M. javanica* and *M. arenaria*. Turbo was resistant to three root-knot nematodes, but Betsy cultivar was susceptible to these species (Table 2). Therefore, Turbo cultivar may provide positive contribution to the management and can be used for infected fields with three *Meloidogyne* species. The reaction of *M. incognita* on Betsy cultivar was statistically different than *M. javanica* and *M. arenaria*. *M. incognita* and *M. javanica* developed slightly on Nirvana cultivar but *M. arenaria* did not develop on this variety and did not produce egg mass or gall on Nirvana (Table 2). These results indicated that the *M. incognita* and *M. javanica* penetrated the roots of the plant but few individuals completed their life cycle. Glaciar cultivar was susceptible to *M. incognita*, *M. javanica* and *M. arenaria.* Glaciar cultivar was more susceptible than Betsy cultivar to root-knot nematodes tested (Table 2).

Table 2. Response of carnation varieties to root-knot nematodes

Nematodes	Carnation variety			
	Standard type		Spray type	
	Turbo	Betsy	Nirvana	Glaciar
Meloidogyne javanica	0.00 a*	2.70 a	0.20 a	5.12 a
Meloidogyne incognita	0.00 a	5.70 b	0.40 a	6.00 a
Meloidogyne arenaria	0.00 a	2.66 a	0.00 a	5.44 a

*Data are means of ten replications. Mean values within a column followed by the same lower case letter are not significantly different (P=0.05) according to Tukey's test.
Plant roots were evaluated according to 0–10 scale proposed by Zeck (Zeck, 1971)
0=no galls, 1=very few small galls, 2=numerous small galls, 3=numerous small galls of which some are grown together, 4=numerous small and some big galls, 5=25% of roots severely galled, 6=50% of roots severely galled, 7=75% of roots severely galled, 8=no healthy roots but plant is still green, 9=roots rotting and plant dying, 10=plant and roots dead.

It is necessary to have knowledge about the reactions of carnation cultivars to *Meloidogyne* species for the management practices. However, there is limited information about the screening of carnation cultivar to root knot nematodes. Fawzy et al. (1991) reported that two carnation cultivars were resistant to *M. incognita*. In another study, Cho et al. (1996) showed that seven, twelve and fourteen carnations were resistant, moderately resistant and susceptible to *M. incognita*, respectively. In this study, we determined that standard carnation variety Turbo was resistant to *M. incognita*, *M. javanica* and *M. arenaria* and spray carnation variety Nirvana was resistant to *M. arenaria*.

Turkey is one of the most significant countries in terms of carnation production. In this study, relationship between nematode species and mostly used carnation cultivars was determined. Resistant carnation cultivars can be effective for the management of RKNs. The data could be used to develop new carnation cultivars, resistant to *Meloidogyne* species in breeding programs. Therefore, more detailed studies are required for development of resistant carnation cultivars against nematodes.

Compliance with Ethical Standards

Conflict of interest

The authors declared that for this research article, they have no actual, potential or perceived conflict of interest.

Author contribution

The contribution of the authors to the present study is equal.

All the authors read and approved the final manuscript. All the authors verify that the text, figures, and tables are original, and they have not been published before.

Ethical approval

Ethics committee approval is not required.

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Data availability

Not applicable.

Consent for publication

Not applicable.

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