

Effect of Different Inoculum Levels of *Meloidogyne incognita*, *Alternaria dauci* and *Rhizoctonia solani* on the Growth, Chlorophyll and Carotenoid Content and Disease Progression of Carrot (*Daucus carota* L.)

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The economic threshold level of *Meloidogyne incognita*, *Alternaria dauci* and *Rhizoctonia solani* were determined on carrot (*Daucus carota* L.) under greenhouse conditions. The results revealed that plant length, plant fresh weight, shoot and root dry weight, chlorophyll and carotenoid decreased progressively with the corresponding increase in the inoculum levels of each pathogen. The significant reduction in plant growth parameters, chlorophyll and carotenoid occurred when 2000 or more second stage juveniles (J2s) of *M. incognita*, 1.0 g or more inoculum of *A. dauci* or *R. solani* per kg soil were inoculated. Maximum reduction in plant growth attributes, chlorophyll and carotenoid occurred at the highest inoculum level of the test pathogens. Increase in the inoculum level of *M. incognita* caused an increase in the number of root galls, while the multiplication of nematode was found inversely related to the inoculum density. The increase in the inoculum levels of *A. dauci* and *R. solani* resulted in a progressive increase in leaf blight and crown rot indices and caused a higher reduction in plant growth parameters. The damaging threshold level of *M. incognita* was 2000 J₂ per kg soil while 1.0 g per kg soil of *A. dauci* or *R. solani* was threshold level on carrot. The assessment of infestation levels of test pathogens will enable growers to cost-effectively select and implement the management tactics.

Keywords: Crown rot, disease indices, galls, leaf blight, root-knot.

Carrot (*Daucus carota* L.) is an important root vegetable grown all over the world. It is a critical source of carotenoids like α -carotene, β -carotene, and phytochemicals such as glutathione, calcium, phosphorus and an excellent source of calcium pectate, an extraordinary pectin fibre that has been known to lower the cholesterol (Kaur et al., 2012). In India, it is cultivated in a total area of 97,000 hectares with an annual production of 1648,000 million tonnes during 2017–2018 (Anonymous, 2018).

Carrot is affected by several pathogens but important pathogens include *Alternaria dauci* (Kuhn) Groves and Skolko cause leaf blight, crown-rot caused by *Rhizoctonia solani* Kuhn (Koike et al., 2006) and root-knot nematode *Meloidogyne incognita* (Kofoid and White) Chitwood. Leaf blight caused by *Alternaria dauci* is probably the most common and destructive disease of carrot worldwide (Pryor and Strandberg, 2001). Initial

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infection occurs on mature leaves where lesions merge resulting in leaf necrosis. As disease progress continues lesions expanded causing leaflets to turn brown, shrivel and die (Agrios, 1997). *Rhizoctonia solani* is a causal organism of damping-off and crown rot of carrot (Grisham and Anderson, 1983). The infection results in damping off seedlings and later crown rot occur on the root. Symptoms show premature senescence and death of foliage in the field. On the roots, dark brown sunken lesions or cankers occur near the crown and other parts of the root. However, root-knot nematodes *Meloidogyne* spp. are one of the key pathogens of carrot all over the world, affecting both quality and quantity of the marketable carrot yield (Sasser and Carter, 1985; Gugino et al., 2006). The infection undergoes several forking and galling in lateral roots as a result of which only a limited amount of water and nutrients absorbed by the plant.

During survey of carrot fields of Aligarh district we found *M. incognita*, *A. dauci* and *R. solani* in most of the field and involved in serious yield losses. Therefore, present study was undertaken to determine effects of different inoculum levels of *M. incognita*, *A. dauci* and *R. solani* on plant growth, chlorophyll, and carotenoid content of carrot.

Materials and Methods

Soil preparation and maintenance of test plants

Loam soil was collected from agricultural fields of Aligarh Muslim University, Aligarh, U.P. India. It was passed through a 10 mesh sieve. Loam soil and cow dung manure were mixed in the ratio (3: 1 v / v) and 15 cm diameter clay pots were filled with 1 kg of the mixture. A small amount of water was poured into each pot to wet the soil surface before sterilization at 137.9 kPa for 20 min. Sterilized pots were allowed to cool down to room temperature before sowing. Carrot cv. Rose Red, seeds were surface sterilized with 0.1% sodium hypochlorite for 2 min and rinsed three times with sterile water. Three sterilized seeds were sown in each pot. One week after germination thinning was done to a single seedling per pot. Un-inoculated plants served as the control and were kept in a glasshouse at 20 ± 2 °C. Pots were arranged in a randomized complete design with each treatment replicated five times. Pots were watered with 200 ml on alternate days.

Preparation of nematode inoculum

The root-knot nematode *Meloidogyne incognita* was collected from the carrot roots and multiplied on the roots of eggplants (*Solanum melongena* L.) using single egg mass. Later, large numbers of egg masses from heavily infected eggplant roots were hand-picked with the help of sterilized forceps. The egg masses were washed with distilled water and placed in a small sieve (9 cm diameter with 1-mm pore size) mounted with crossed layers of tissue paper. The sieve was placed in a Petri plate containing distilled water deep enough to contact the egg masses. A number of these assemblies were kept in an incubator running at 25 ± 1 °C in order to obtain the required number of second-stage juveniles. The hatched second-stage juveniles were collected from the Petri plates every 24 h, fresh water was added to repeat the process. For counting nematode juveniles, an average of 5 counts was made to determine the density of nematodes in the suspension. The volume

of nematode suspension was so adjusted that each ml may contain 200 ± 5 nematodes. In the experiment, 5 ml, 10 ml, 20 ml and 40 ml suspensions were inoculated in order to get 1000, 2000, 4000, and 8000 freshly hatched second stage juveniles of *M. incognita*.

Isolation and preparation of fungal inoculum

Carrot plants showing blight and crown-rot symptoms were collected in polythene bags from infected fields. Washing with sterilized distilled water were done to isolate fungus from infected leaves and roots of carrot. Later, the infected leaves / roots were cut into approximately 5 mm square pieces and transferred to Petri dish containing 0.1% sodium hypochlorite solution as described for seed sterilization above. After one minute leaf / root pieces were washed at least three times in distilled water and dried on blotting paper. Infected root / leaf pieces were then plated separately in petri plates containing potato dextrose agar (PDA) medium with the help of sterilized forceps under aseptic condition. Petri plates were then incubated at 28 ± 2 °C for 15 days. The fungus that developed on leaf / root segments were examined and identified. The identity of the fungus was confirmed as *A. dauci* and *R. solani* using microscopic morphological characteristics.

For obtaining sufficient inoculum of fungi, Richard's liquid medium was used (10 g potassium nitrate; 5 g potassium dihydrogen phosphate; 2.5 g magnesium sulphate; 0.02 g ferric chloride; 50 g sucrose, and 1000 ml distilled water (Riker and Riker, 1936). The Richards liquid medium was prepared and filtered through muslin cloth, sterilized in an autoclave at 103.4 kPa for 15 min in 250 mL Erlenmeyer flasks containing 80 ml of liquid medium. Both fungi were inoculated separately with a sterile inoculation needle in to each flask, were incubated at 28 ± 2 °C for about 15 days. After sufficient fungal growth the liquid medium was filtered through Whatman filter paper No. 1. The fungal mycelia mat on the filter paper was washed in distilled water and excess water and nutrients removed with help of blotting paper. Ten g fungal mycelium was placed in 100 ml of distilled water and blended (10,000 rpm) for 30 sec in a Waring blender. The 5, 10, 20, and 40 ml of this suspension containing 0.5, 1.0, 2.0, and 4.0 g fungus was inoculated.

Inoculation technique

Two week old carrot seedlings were inoculated with the test pathogens. Feeder roots of seedlings were exposed just before inoculation by carefully removing the top layer of soil and required quantity of inoculum was poured uniformly all around the exposed root using sterilized pipette. Exposed roots were covered by levelling the soil properly. Each treatment was replicated five times and un-inoculated plants served as control. Experiment was terminated after 90 days of inoculation.

Observations

The plants were harvested 90 days after inoculation. Data on plant length, plant fresh weight, shoot dry weight, root dry weight, chlorophyll, carotenoid content, number of galls / root system, and nematode population were recorded. Crown-rot and blight indices were also recorded. The length of plants was recorded in cm from the top of the

first leaf to end of the root. Excess water was removed by blotting before weighing the plant for fresh weight. The plants were cut with a knife above the base of the root emergence zone to separate shoot and root. Shoots and roots were kept in envelopes at 60 °C for 2–3 days before weighing for dry weight. A 250 g subsample of well-mixed soil from each treatment was processed by Cobb's sieving and decanting technique followed by Baermann funnel extraction (Southey, 1986). Nematode suspension was collected after 24 h and the numbers of nematodes were counted in five aliquots of 1 ml of suspension from each sample. The means of five counts were used to calculate the population of nematodes per kg soil. To estimate the number of juveniles, eggs and females inside the roots, a 1 g subsample of roots was macerated in a Waring blender and counts were made from the suspension thus obtained. Numbers of nematodes present in roots were calculated by multiplying the number of nematodes present in 1 g of root by the total weight of root. Final nematodes population was presented in the manuscript by adding the nematodes present in 1 kg soil with the nematodes present in a root. Final nematode population was calculated by combining soil and root data for each sample.

Disease index

Leaf blight and crown rot index was determined by scoring the severity of disease on visual observations of disease symptoms. Crown rot symptoms were observed on roots while blight symptoms were observed on leaves. Disease rating was done on a scale ranging from 0 (no disease) to 5 (severe rot / blight) (Nesha and Siddiqui, 2013).

Estimation of chlorophyll and carotenoid content

The chlorophyll and carotenoid content in the fresh leaf samples was estimated by the method of Mackinney (1941). One g of freshly cut leaves was ground to fine pulp using a mortar and pestle after pouring 20 cm³ of 80% acetone. The mixture was centrifuged at 5,000 rpm for 5 minutes. The supernatant was collected in 100 cm³ volumetric flask. The residue was washed three times, using 80% acetone. Each washing was collected in the same volumetric flask and volume was made up to mark, using 80% acetone. The absorbance was read at 645 and 663 nm for chlorophyll and 480 and 510 nm for carotenoid against the blank (80% acetone) on spectrophotometer (Shimadzu UV-1700, Tokyo, Japan).

Statistical analysis

One way ANOVA was used to test the significance ($p \leq 0.05$) of data, i.e., plant length, plant fresh weight, shoot and root dry weight, chlorophyll and carotenoid contents. Least significant differences were calculated at $P=0.05$. Duncan's new multiple range test was employed to show significant differences between the treatments. Graphs of number of galls per root system and nematode population were prepared using Sigma Plot™. Error bars indicate the standard error.

Results

Effects on plant growth

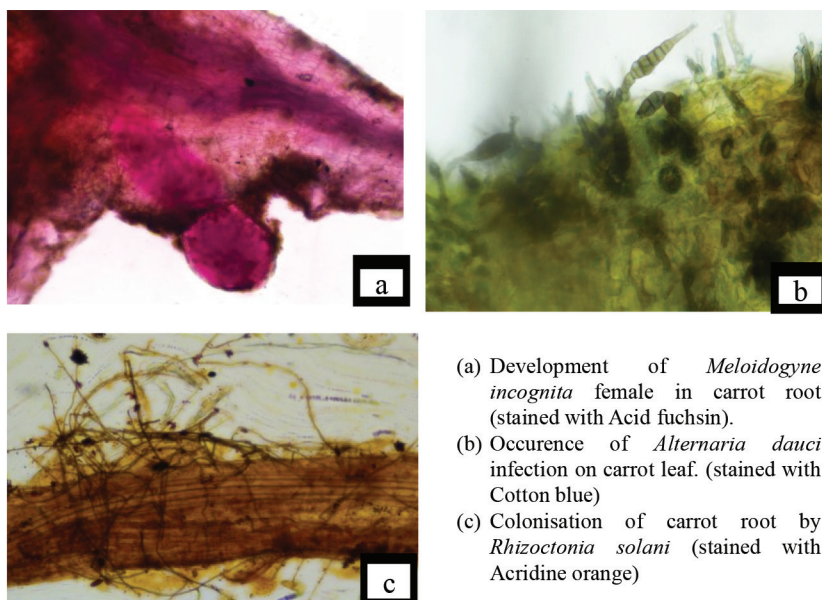
A gradual decrease in plant growth parameters viz., plant length, plant fresh weight, shoot dry weight, and root dry weight was observed with the corresponding increase in the inoculum level of each pathogen, i.e. *M. incognita*, *A. dauci* and *R. solani* (Table 1). However, plant growth reduced significantly over control when 2000 or more second stage juveniles of *M. incognita*, 1.0 g or more inoculum of *A. dauci* or *R. solani* per kg soil were inoculated. Maximum reduction in plant growth was observed at the highest inoculum of the test pathogens. Among tested pathogens, *R. solani* caused greater reduction in plant growth, followed by *A. dauci* and *M. incognita* with varying degree of infection (Table 1, Fig. 1).

Inoculation of 2000 juveniles of *M. incognita* caused statistically significant reduction in plant length (9.68%), plant fresh weight (23.69%), shoot dry weight (22.97%), and root dry weight (39.22%) of carrot (Table 1). Highest reduction was recorded in plant length (23.04%), plant fresh weight (34.02%), shoot dry weight (47.37%) and root dry weight (57.24%) caused by inoculation of 8000 juveniles of *M. incognita* (Table 1).

Table 1
 Effect of different inoculum levels of *Meloidogyne incognita*, *Alternaria dauci* and *Rhizoctonia solani* on the growth, chlorophyll, carotenoid and disease index of carrot

Treatments		Plant length (cm)	Plant fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)	Total chlorophyll (mg/g fw)	Carotenoid content (mg/g fw)	Blight/rot index
<i>M. incognita</i>	Control	52.70 a	62.90 a	2.09 a	2.83 a	0.273 a	0.0507 a	–
	1000 J ₂	49.12 ab	58.33 a	1.86 a	2.50 a	0.258 a	0.0496 ab	–
	2000 J ₂	47.60 b	48.00 b	1.61 b	1.72 b	0.240 b	0.0485 bc	–
	4000 J ₂	43.40 c	44.12 bc	1.28 c	1.34 c	0.226 bc	0.0469 c	–
	8000 J ₂	40.56 c	41.50 c	1.10 c	1.21 c	0.215 c	0.0447 d	–
P ≤ 0.05		3.61	4.57	0.23	0.34	0.015	0.0018	
<i>A. dauci</i>	Control	52.70 a	62.90 a	2.09 a	2.83 a	0.273 a	0.0507 a	0
	0.5 g	47.94 a	57.10 a	1.75 a	2.39 a	0.246 a	0.0483 ab	2
	1.0 g	39.30 b	37.05 b	1.38 b	1.56 b	0.218 b	0.0470 bc	3
	2.0 g	36.34 bc	33.50 bc	1.12 bc	1.22 bc	0.201 bc	0.0453 cd	4
	4.0 g	33.60 c	29.30 c	0.97 c	1.09 c	0.185 c	0.0436 d	5
P ≤ 0.05		4.77	5.86	0.34	0.44	0.027	0.0024	–
<i>R. solani</i>	Control	52.70 a	62.90 a	2.09 a	2.83 a	0.273 a	0.0507 a	0
	0.5 g	47.10 a	56.30 a	1.66 a	2.30 a	0.238 ab	0.0476 ab	2
	1.0 g	36.50 b	34.18 b	1.08 b	1.26 b	0.205 bc	0.0455 bc	3
	2.0 g	33.30 bc	30.00 bc	0.82 bc	1.01 bc	0.183 c	0.0431 cd	4
	4.0 g	30.48 c	27.20 c	0.61 c	0.70 c	0.169 c	0.0411 d	5
P ≤ 0.05		5.60	6.61	0.43	0.53	0.035	0.0032	–

Data are mean of 5 replicates. The mean values within a column followed by the different letter are significantly different at p=0.05 by Duncan's new multiple range test (DMRT).



- (a) Development of *Meloidogyne incognita* female in carrot root (stained with Acid fuchsin).
 (b) Occurrence of *Alternaria dauci* infection on carrot leaf. (stained with Cotton blue)
 (c) Colonisation of carrot root by *Rhizoctonia solani* (stained with Acridine orange)

Fig. 1. Infection of *M. incognita*, *A. dauci* and *R. solani* on carrot

Similarly, inoculation of 1.0 g of *A. dauci* caused significant reduction in plant length (25.43%), plant fresh weight (41.10%), shoot dry weight (33.97%) and root dry weight (44.88%) (Table 1). However, inoculation of 4.0 g of *A. dauci* caused a maximum reduction in plant length (36.24%), plant fresh weight (53.42%), shoot dry weight (53.59%) and root dry weight (61.48%) of carrot (Table 1).

Plants inoculated with 1.0 g of *R. solani* showed a significant reduction in plant length (30.74%), plant fresh weight (45.66%), shoot dry weight (48.33%) and root dry weight (55.48%) (Table 1). However, inoculation of 4.0 g of *R. solani* caused highest reduction in plant length (42.16%), plant fresh weight (56.76%), shoot dry weight (70.81%) and root dry weight (75.27%) (Table 1).

Effects on chlorophyll and carotenoid content

Plants inoculated with 2000 juveniles of *M. incognita* / kg soil caused significant reduction in chlorophyll and carotenoid content as compared to control. The greater reduction in chlorophyll and carotenoid content was recorded in plants inoculated with 8000 juveniles of *M. incognita* / kg soil followed by 4000 and 2000 juveniles. However, inoculation of 1000 juveniles of *M. incognita* caused non-significant reduction in chlorophyll and carotenoid content (Table 1).

Similarly, a significant reduction over control in chlorophyll and carotenoid of *A. dauci* inoculated plants was observed when 1.0 g or more *A. dauci* / kg soil was inoculated. Inoculation of 4.0 g of *A. dauci* caused the highest reduction in chlorophyll and carotenoid content followed by 2.0 g and 1.0 g of *A. dauci* / kg soil. However, inoculation of 0.5 g of *A. dauci* / kg soil caused non-significant reduction in chlorophyll and carotenoid content (Table 1).

Inoculation of *R. solani* also caused significant reduction in chlorophyll and carotenoid content when 1.0 g or more *R. solani* inoculum / kg soil was inoculated. Inoculation of 4.0 g *R. solani* inoculum / kg soil caused higher reduction in chlorophyll and carotenoid content followed by 2.0 and 1.0 g inoculum. Inoculation of 0.5 g of *R. solani* inoculum / kg soil caused non-significant reduction in chlorophyll and carotenoid content (Table 1).

Effects on root galling and nematode multiplication

An increase in the number of root galls per root system was observed with the increase in the inoculum level of nematode. The higher root galling was observed at the highest level (8000 juveniles) of inoculum while inoculation of 1000 juveniles of *M. incognita* caused a lowest root galling (Fig. 2a). The multiplication of *M. incognita* was found density dependent. Maximum multiplication of the nematode was recorded at the lowest inoculum level (1000 juveniles), and minimum multiplication at the highest inoculum (8000 juveniles) of *M. incognita* (Fig. 2b). Although the total number of nematodes was the highest in samples treated with the top inoculum concentration, the reproductive rate was in fact the lowest in these samples.

Effects on blight and crown-rot index

Blight and crown-rot indices caused by *A. dauci* and *R. solani* respectively were also found to increase with the inoculum level of each pathogen. Leaf blight indices were recorded 2, 3, 4, and 5 respectively at 0.5, 1.0, 2.0 and 4.0 g of inoculum level of *A. dauci*. Similarly, crown rot indices were also 2, 3, 4, and 5 at 0.5, 1.0, 2.0 and 4.0 g of inoculum level of *R. solani* (Table 1).

Discussion

All the inoculum levels of *M. incognita* caused reduction in the carrot growth, chlorophyll and carotenoid content and carrot quality, but significant reductions were noticed when 2000 or more juveniles of *M. incognita* per kg soil were inoculated. The reduction in carrot marketability rather than total quantity of yield due to infections by *M. incognita* has been reported previously (Huang and Charchar, 1982). However, a progressive decrease in chlorophyll content with increase in inoculum level of nematode on different crops was reported (Shukla and Haseeb, 1998; Kheir et al., 2004). Severity in root galling caused by *M. incognita* was found to be increased with the increase in inoculum level. This progressive increase in the number of root galls with increased inoculum densities of *M. incognita* have also been reported on different crops (Agwu and Ezigbo, 2005; Anwar et al., 2007; Vovlas et al., 2008). The multiplication of *M. incognita* in carrot root was found density-dependent. The reduction in the reproductive rate of nematode at higher inoculum densities has been previously reported for plants infected with nematodes (Pathak et al., 2000; Ganaie and Khan, 2011). The higher nematode multiplication at lower inoculum levels might be due to the lesser competition for food and space than at higher inoculum levels.

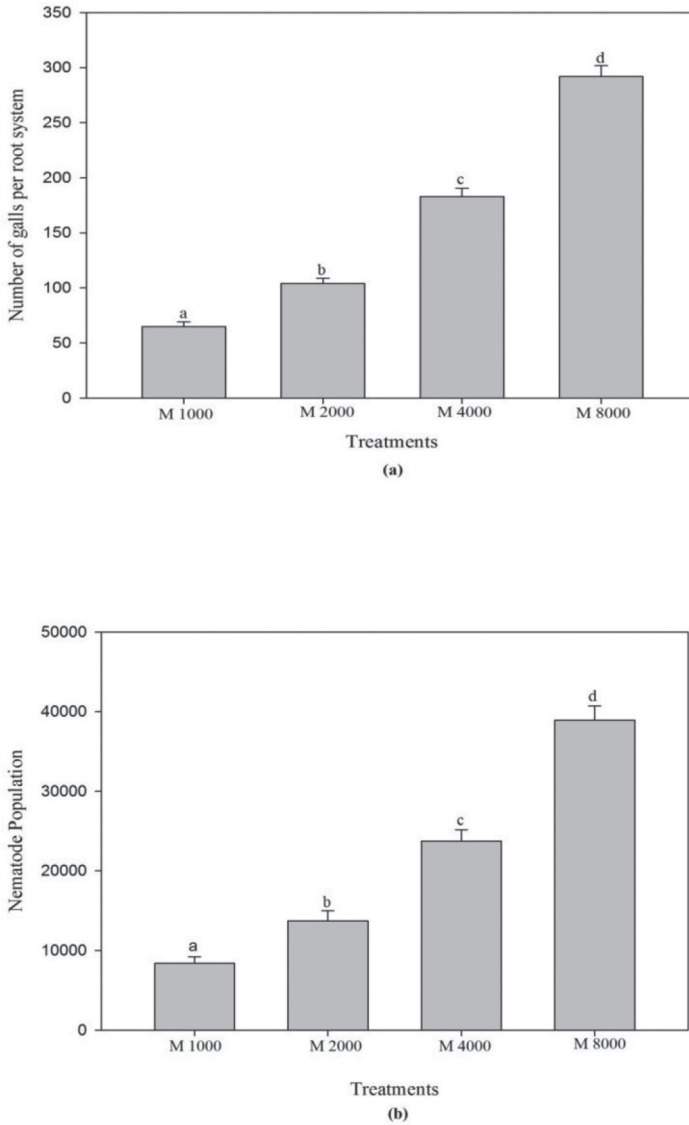


Fig 2. (a). Effects of different inoculum levels of *Meloidogyne incognita* (M) on galling of carrot.
 (b). Effects of different inoculum levels of *Meloidogyne incognita* (M) on nematode multiplication of carrot

The statistically significant reduction in plant growth, chlorophyll and carotenoid content was observed at or above 1.0 g of *A. dauci* or *R. solani* per kg soil. *Alternaria* leaf blight can considerably reduce leaf photosynthetic activity and necrotic foliage, which contribute to a significant reduction in carrot yield, chlorophyll and carotenoid contents (Boedo et al., 2012). Dugdale et al. (2000) applied specific bioassay on detached leaves of carrot where chlorophyll concentrations declined as disease progressed. Reduction of leaf surface caused by disease also prevents root development (Ellis, 1971). Under favourable

conditions, the leaf lesions ultimately coalesce, giving the leaf tissue a blighted appearance, thus reducing the photosynthetic activity (Farrar et al., 2004). On the other hand, *R. solani* cause of damping off and crown-rot of carrot was found most damaging under greenhouse condition. Roots were severely rotted and colonised abundantly by *Rhizoctonia* hyphae at higher inoculum levels (Walker, 1991). The direct relationship between inoculum level and disease severity due to infection caused by *R. solani* on lima bean and cabbage has been reported by Warren (1975) and Keinath (1995), respectively.

The damaging threshold of *M. incognita* was found 2000 or more second stage juveniles of *M. incognita* per kg soil, and 1.0 g or more *A. dauci* or *R. solani* inoculum per kg soil. Research on assessing soil nematode and fungi infestation levels will enable growers to cost-effectively select and implement the management tactics available against test pathogens not only in carrot fields but in fields containing other susceptible vegetable crops and to also implement a whole farm management strategy for test pathogens.

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