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Effects of *Meloidogyne incognita*, *Alternaria dauci* and *Fusarium solani* on Carrot in Different Types of Soil

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Effects of *Meloidogyne incognita*, *Alternaria dauci* and *Fusarium solani* were studied on carrot (*Daucus carota* L.) growth, chlorophyll, carotenoid and proline contents in different types of soil. Plants grown in 20:80 and 40:60% sand:clay soil mixtures showed a significant increase in root dry weight, chlorophyll and carotenoid contents compared to plants grown in 100% clay soil. However, use of 60:40 sand:clay resulted in a similar root dry weight, chlorophyll and carotenoid contents as was found in carrots grown in 100% clay soil. Inoculation of plants with *M. incognita, A. dauci* or *F. solani* caused a significant reduction in root dry weight, chlorophyll and carotenoid contents in all soil types as compared to their respective control. Inoculation of plants by *A. dauci* caused the greatest reduction in root dry weight followed by *F. solani* and *M. incognita* in different sand and clay mixtures. Use of 20:80, 40:60 or 60:40 sand:clay mixtures caused a significant increase in proline content of plants over those grown in 100% clay soil. Similarly, inoculation of *M. incognita, A. dauci* and *F. solani* caused a significant increase in proline content in all soil types compared to their respective control.

Keywords: Carotenoids, chlorophyll, clay, proline, sand.

Carrot (*Daucus carota* L.) is one of the popular vegetables in many countries and had important nutritional value (Al-Harbi et al., 1997). It is being consumed mainly due to their pleasant flavour and perceived health benefits related to minerals, fibre and vitamins including vitamin B and beta carotene that promotes the synthesis of vitamin A. Carrot is an excellent source of iron, calcium, phosphorus, folic acid and sugars (Yawalker, 1992).

Various plant pathogens cause damage on carrot. Important fungal diseases of carrot includes Alternaria leaf blight caused by *Alternaria dauci*; black root rot by *Thielaviopsis basicola*; black rot by *Alternaria radicina*; cavity spot by *Pythium* spp.; Cercospora leaf spot by *Cercospora carotae*; cottony rot by *Sclerotinia sclerotiorum* and root rot by *Fusarium* spp (Koike et al., 2006). Among nematodes, root-knot nematode species *Meloidogyne hapla, M. javanica* and *M. incognita* are of worldwide economic importance for carrot cultivation (Abawi et al., 2009) and impacting both the quantity and quality of marketable carrot yield (Sasser and Carter, 1985). Attacks by *M. javanica* and *M. incognita* prevail in tropical and sub-tropical areas of the world (Rubatzky et al., 1999).

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Soil type and texture is recognized as an important factor that affects both crop productivity and plant pathogens including plant parasitic nematode communities (Koenning and Barker, 1995). Soil texture largely determines soil moisture holding capacity and aeration and has impacts on the nematode's ability to hatch, move through soil, locate and penetrate a host, and mate (Koenning and Barker, 1995). Thus, soil type influences the damage potential of several nematodes (Koenning et al., 1988). Soils that hold generous amount of water are less subject to leaching losses of nutrients. After a soil is saturated with water, all of the excess water and some of the nutrients that are in soil solution are leached downward in soil profile. In addition, soil texture is a reflection of the particle size and small particles (clay and silt) have a much larger surface area than the larger sand particles. This large surface area allows the soil to hold a greater quantity of water.

During the course of survey of carrot fields of Aligarh district for plant parasitic nematodes and fungi, we found frequent occurrence of *M. incognita* (Kofoid and White) Chitwood, *A. dauci* (J.G. Kühn) Rostrup and *F. solani* (Martius) Sacc. in different fields having different type of soil. It was thought desirable to study the effect of these pathogens individually and carrot growth, chlorophyll, carotenoids and proline contents in different soils types.

Materials and Methods

Plant culture

Soil mixtures containing clay soil and sand in the ratio of 100% clay soil, 80:20 clay:sand, 60:40 clay:sand and 40:60 (v/v) clay:sand mixtures were prepared and autoclaved. Later, the mixtures were fertilized with inorganic fertilizers at the rate of 0.03 g N, 0.04 g K and 0.05 g P per kg soil. Seeds of carrot cv. Rose red were surface sterilized with 0.01% mercuric chloride for 2 minutes and washed three times in distilled water. Sowing of seeds was done in different soil mixtures placed in 15-cm pots. One week after germination thinning was done to maintain single plant per pot. Two days after thinning seedlings were inoculated with *Meloidogyne incognita, Alternaria dauci* and *Fusarium solani*. Each treatment was replicated five times. Pots were placed on a glass house bench maintained at 25 °C. Pots were watered as needed and experiment was terminated 90 days after inoculation.

Nematode inoculum

M. incognita was collected from carrot field and multiplied on egg plant (*Solanum melongena* L.) using a single egg-mass from a single female. Egg-masses were hand-picked using sterilized forceps and placed in 9-cm diameter sieves of 1-mm pore size which were previously mounted with cross layered tissue paper for hatching. These sieves were placed in Petri dishes containing distilled water and kept in an incubator at 27 °C. In the experiment, 2000 freshly hatched second stage juveniles of *M. incognita* were inoculated as described below.

Maintenance and preparation of fungal inocula

Pure cultures of *F. solani* and *A. dauci* were obtained from the Division of Mycology and Plant Pathology, Indian Agricultural Research Institute, New Delhi. The cultures were maintained on Petri dishes containing potato dextrose agar media and incubated in an incubator at 25 ºC. For obtaining sufficient inoculums, *F. solani* and *A. dauci* were cultured separately on Richards liquid medium (Riker and Riker, 1936) at 25 ± 1 ºC for 15 days. After sufficient growth of fungus, the liquid medium was filtered through Whatman filter paper 1. The mat of fungal mycelium was washed in distilled water and was collected on blotting sheets to remove excess of water and nutrients. The inoculum was prepared by mixing 10 g fungal mycelium in 100 ml distilled water for 30 s in a Waring blender. Ten ml of this suspension containing 1 g of fungus was used as inoculum. The inoculated plants were then transferred to glasshouse benches, watered regularly and assessed for the development of the disease.

Inoculation techniques

Soil around the roots was carefully removed and suspensions of *M. incognita*, *A. dauci* and *F. solani* were poured around the roots uniformly and soil replaced. In control pots, water was poured in similar amount to inoculum suspension. The four soil types were inoculated with *M. incognita*, *A. dauci* and *F. solani* alone and a control. There were 16 treatments, i.e. 4 types of soil × 4 treatments including control. Each treatment was replicated five times, i.e. 16×5 = 80 pots in a complete factorial design.

Observations

The plants were harvested 90 days after inoculation. Data on plant length, plant fresh weight, dry weight, number of galls, nematode population, chlorophyll, carotenoids and proline contents were recorded. Root rot and blight indices and nematode population 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 ºC for 2–3 days before weighing the plants 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. Root rot and blight index was determined by scoring the severity of disease on visual observations of disease symptoms. Root rot symptoms were observed on roots while blight

symptoms were observed on leaves. Disease rating was done on a scale ranging from 0 to 5 scale. Rotting symptoms were observed on the root where 0 = no disease; 1 = rotting symptoms up to 12.5% on root; 2 = rotting 12.6 to 25% on root; 3 = rooting 25.1 to 37.5 on root; 4 = 37.6 to 50% on root; 5 = more than 50% rooting on the root system. Similarly, blight symptoms were observed on the leaves where 0 = no disease; 1 = blight symptoms up to 12.5 leaves; 2 = blight 12.6 to 25% on leaves; 3 = blight 25.1 to 37.5 on leaves; 4 = blight 37.6 to 50% on leaves; 5 = more than 50% blight symptoms on leaves. The chlorophyll content and carotenoids in fresh leaves were estimated following the method of Mackinney (1941). The proline content in the fresh leaf samples was measured by the method of Bates et al. (1973).

Statistical analysis

Data obtained were analysed statistically by analysis of variance using http://hau.ernet.in/about/opstat.php (off campus user). The critical differences (C.D.) were calculated at $P \leq 0.05$. Duncan's multiple range test was employed to denote significant differences between the treatments. Means and standard errors of five replicates are given.

Results

Root dry weight

Plants grown in 20:80 and 40:60% sand:clay mixture showed a significant increase in root dry weight over plants grown in 100% clay soil (Table 1). However, use of 60:40 sand:clay resulted in a similar root dry weight as was found in plants grown with 100% clay. Inoculation with *M. incognita, A. dauci* or *F. solani* caused a significant reduction in root dry weight in all soil types as compared to their respective control. Inoculation with *M. incognita, A. dauci* or *F. solani* in 100% clay soil caused statistically a similar reduction in root dry weight. However, inoculation of *A. dauci* caused the greatest reduction in root dry weight followed by *F. solani* and *M. incognita* in different sand and clay mixtures. Root dry weight of *M. incognita, A. dauci* or *F. solani*-inoculated plants grown on 40:60 sand:clay mixture was statistically similar to that grown with 60:40 sand:clay (Table 1).

Chlorophyll content

Plants grown in 20:80 and 40:60% sand:clay mixture caused a significant increase in chlorophyll content compared to plants grown in 60:40 sand and clay mixture respectively (Table 2). However, mixture of 60:40 sand:clay resulted in similar chlorophyll content as was found in plants grown in 100% clay soil. Inoculation with *M. incognita, A. dauci* or *F. solani* caused a significant reduction in chlorophyll content as compared to their respective control. However, *A. dauci* caused a greater reduction in chlorophyll content than *F. solani* or *M. incognita* in different sand and clay mixtures. Chlorophyll

Table 1

Effects of *Meloidogyne incognita*, *Alternaria dauci* and *Fusarium solani* in different types of soil on the growth of carrot

Treatments	Soil type	Plant length (cm)	Plant fresh weight (g)	Shoot dry weight (g)	Root dry weight (g)
Control	Clay soil	$52.02^{e} \pm 0.694$	$63.9^{d} \pm 0.524$	$2.03^{de} \pm 0.066$	$2.95^{bc} \pm 0.069$
	Clay soil + 20% sand	$70.20^{a} \pm 0.456$	$69.42^{a} \pm 0.535$	$3.20^{a} \pm 0.122$	$3.40^{a} \pm 0.149$
	Clay soil + 40% sand	$69.50^{a} \pm 0.327$	$67.30^{b} \pm 0.363$	$2.79^{b} \pm 0.251$	$3.31^{a} \pm 0.097$
	Clay soil + 60% sand	$54.44^{cd} \pm 0.369$	$65.40^{c} \pm 0.241$	$2.41^{c} \pm 0.060$	$3.16^{ab} \pm 0.076$
Meloidogyne incognita	Clay soil	$48.60^{fg} \pm 0.298$	$47.50^{f} \pm 0.472$	$1.80^{efg} \pm 0.088$	$1.44^{h} \pm 0.043$
	Clay soil + 20% sand	$60.20^{b} \pm 0.928$	$49.80^{e} \pm 0.378$	$2.25^{cd} \pm 0.083$	$2.85^{c} \pm 0.148$
	Clay soil + 40% sand	$56.00^{c} \pm 0.467$	$48.70^{ef} \pm 0.613$	$2.08^{de} \pm 0.104$	$3.01^{bc} \pm 0.089$
	Clay soil + 60% sand	$49.48^{f} \pm 0.411$	$47.90^{f} \pm 0.376$	$1.85^{ef} \pm 0.080$	$2.84^{c} \pm 0.081$
Alternaria dauci	Clay soil	$39.42^{i} \pm 0.672$	$35.20^{k} \pm 0.274$	$0.91^{k} \pm 0.066$	$1.19^{h} \pm 0.041$
	Clay soil + 20% sand	$54.70^{cd} \pm 0.212$	$42.50^{h} \pm 0.415$	$1.45^{hij} \pm 0.083$	$2.15^{ef} \pm 0.038$
	Clay soil + 40% sand	$53.50^{d} \pm 0.511$	$40.80^{j} \pm 0.207$	$1.35^{ij} \pm 0.059$	$2.05^{fg} \pm 0.047$
	Clay soil + 60% sand	$45.80^{h} \pm 0.507$	$38.40^{j} \pm 0.354$	$1.16^{ijk} \pm 0.075$	$1.87^{g} \pm 0.165$
Fusarium solani	Clay soil	$45.40^{h} \pm 0.635$	$38.40^{j} \pm 0.395$	$1.27^{ij} \pm 0.049$	$1.30^{h} \pm 0.037$
	Clay soil + 20% sand	$59.40^{b} \pm 0.281$	$45.12^{g} \pm 0.221$	$1.68^{fgh} \pm 0.087$	$2.51^{d} \pm 0.068$
	Clay soil + 40% sand	$55.70^{c} \pm 0.581$	$42.90^{h} \pm 0.756$	$1.51^{ghi} \pm 0.051$	$2.39^{de} \pm 0.093$
	Clay soil + 60% sand	$47.70^{g} \pm 0.580$	$40.12^{i} \pm 0.331$	$1.32^{ij} \pm 0.082$	$2.19^{ef} \pm 0.058$
C.D. $P \leq 0.05$		1.48	1.21	0.28	0.25

* Means ± standard error of five replicates are shown

* Values in a column followed by the different letters are significantly different at $P \leq 0.05$ using Duncan's multiple range test

* C.D. = Critical difference

contents of *M. incognita, A. dauci* or *F. solani*-inoculated plants grown in 20:80 sand: clay mixture was statistically similar to that grown in 40:60 sand:clay (Table 2).

Carotenoid content

Plants grown 20:80 and 40:60% sand:clay mixture caused a significant increase in carotenoid content compared to plants grown in clay soil (Table 2). Inoculation with *M. incognita*, *A. dauci* and *F. solani* caused a significant reduction in carotenoid content in all soil types as compared to their respective control. The one exception was the effect of *F. solani* in 100% clay soil. *A. dauci* and *M. incognita* caused the greatest reduction in carotenoid content followed by *F. solani* in different sand and clay mixtures. Carotenoid contents of *M. incognita* or *A. dauci* inoculated plants were statistically similar to each other in either of the four soil types (Table 2).

Proline content

Addition of sand to clay soil caused a significant increase in proline content (Table 2). Increase in proline content was the greatest in plants grown in 60:40 sand:clay followed by 40:60 and 20:80% sand:clay. Inoculation with *M. incognita, A. dauci* and *F. solani* caused a significant increase in proline content in all soil types compared to their respective control. *A. dauci* caused the greatest increase in proline content followed by *F. solani* and *M. incognita* (Table 2).

Root galling and nematode multiplication

Root galling and nematode multiplication was the greatest in plants grown in 40:60 sand and clay mixture followed by 60:40 sand:clay and 20:80 sand:clay mixture (Table 2). Least galling intensity and nematode multiplication was observed in plant grown in 100% clay soil.

Disease indices

Blight and root-rot indices were 3 when *A. dauci* and *F. solani* inoculated plants were grown in 100% clay soil (Table 2). Indices were observed 2 when *A. dauci* and *F. solani* were inoculated in other soil types.

Discussion

Soil characteristics play an important role in the plant's ability to extract water and nutrients. The soil must provide a satisfactory environment for plant to grow to their potential. Carrots like loose rich soil, preferably a little sandy. Loose sandy soil allows growing long slender straight carrots. Moreover, soil should be well drained and loose to prevent forking and stunting of the root growth. The highest growth of carrot, greater chlorophyll and carotenoid contents was observed in the 80:20 clay:sand mixture and may be due to optimum pore space, water holding capacity or aeration of the soil (Black, 1973).

Damage caused by nematodes is often proportionally greater in sandy soils where nematodes can move more freely, than in heavier soils where movement is impeded (Ravichandra, 2014). Adequate soil moisture is also essential for free movement to infect roots, therefore, plant growth, chlorophyll and carotenoid contents were also equally affected in different types of soils by nematode parasitism (Trudgill and Phillips, 1997). However, highest nematode multiplication was observed in 40:60 sand:clay mixture. High rate of nematode multiplication in 40:60 sand:clay may partly be due to higher content of coarse particles in the soil (Koenning et al., 1988). Increased pore size and soil characteristics are favourable for nematode movement and multiplication (Wallace, 1963). On the other hand, pure clay has unfavourable pore size and aeration which probably resulted in poor nematode multiplication and movement (Young and Heatherly, 1990). Low multiplication of nematodes in 60:40 sand:clay may be due to low water holding capacity (Wallace, 1963).

Table 2

Effects of *Meloidogyne incognita*, *Alternaria dauci* and *Fusarium solani* in different types of soil on chlorophyll, carotenoid, proline contents, nematode galling and disease index

Treatments	Soil type	Total chlorophyll (mg/g)	Carotenoid content (mg/g)	Proline content (μmol/g)	No. of galls/root	Nematode population/kg soil	Blight/ root–rot index
Control	Clay soil	$0.278^{cd} \pm 0.004$	$0.0508^{ef} \pm 0.0010$	$0.0761^{k} \pm 0.0014$	–	–	–
	Clay soil + 20% sand	$0.326^{a} \pm 0.005$	$0.0656^{a} \pm 0.0011$	$0.0831^{j} \pm 0.0006$	–	–	–
	Clay soil + 40% sand	$0.312^{b} \pm 0.004$	$0.0625^{b} \pm 0.0013$	$0.0890^{i} \pm 0.0006$	–	–	–
	Clay soil + 60% sand	$0.282^{cd} \pm 0.004$	$0.0540^{c} \pm 0.0010$	$0.0949^{gh} \pm 0.0006$	–	–	–
Meloidogyne incognita	Clay soil	$0.256^{fg} \pm 0.005$	$0.0478^{gh} \pm 0.0009$	$0.0969^{fg} \pm 0.0014$	$81^{c} \pm 6.34$	$10530^{d} \pm 493.9$	–
	Clay soil + 20% sand	$0.283^{c} \pm 0.004$	$0.0507^{ef} \pm 0.0010$	$0.0938^{h} \pm 0.0005$	$108^{b} \pm 4.14$	$14340^{c} \pm 374.8$	–
	Clay soil + 40% sand	$0.272^{cde} \pm 0.003$	$0.0505^{ef} \pm 0.0015$	$0.1046^{c} \pm 0.0008$	$142^{a} \pm 5.14$	$18670^{a} \pm 320.5$	–
	Clay soil + 60% sand	$0.269^{def} \pm 0.003$	$0.0483^{gh} \pm 0.0006$	$0.1116^{d} \pm 0.0007$	$122^{b} \pm 5.55$	$15690^{b} \pm 340.5$	–
Alternaria dauci	Clay soil	$0.222^{i} \pm 0.005$	$0.0473^{h} \pm 0.0011$	$0.1039^{e} \pm 0.0010$	–	–	3
	Clay soil + 20% sand	$0.269^{def} \pm 0.005$	$0.0506^{ef} \pm 0.0014$	$0.1147^{c} \pm 0.0007$	–	–	2
	Clay soil + 40% sand	$0.252^{gh} \pm 0.004$	$0.0492^{fgh} \pm 0.0006$	$0.1257^{b} \pm 0.0009$	–	–	2
	Clay soil + 60% sand	$0.241^{h} \pm 0.006$	$0.0486^{gh} \pm 0.0013$	$0.1347^{a} \pm 0.0008$	–	–	2
Fusarium solani	Clay soil	$0.251^{gh} \pm 0.004$	$0.0496^{efg} \pm 0.0012$	$0.0985^{f} \pm 0.0017$	–	–	3
	Clay soil + 20% sand	$0.273^{cde} \pm 0.005$	$0.0531^{cd} \pm 0.0007$	$0.1062^{e} \pm 0.0008$	–	–	2
	Clay soil + 40% sand	$0.262^{efg} \pm 0.004$	$0.0517^{de} \pm 0.0010$	$0.1131^{cd} \pm 0.0010$	–	–	2
	Clay soil + 60% sand	$0.258^{fg} \pm 0.005$	$0.0496^{efg} \pm 0.0011$	$0.1270^{b} \pm 0.0006$	–	–	2
C.D. $P \leq 0.05$		0.012	0.0021	0.0027	16.2	1174.2	–

*Means ± standard error of five replicates are shown

*Values in a column followed by the different letters are significantly different at $P \leq 0.05$ using Duncan's multiple range test

*C.D.=Critical difference

In the present study we found greater incidence of blight and root rot incidence in

clay soil than in the mixtures of sand and clay. Similarly, Kuldhar et al. (2013) observed greater wilt incidence in clay soil followed by sandy loam and sandy soil. In contrast, Sagar and Sugha (1998) reported high pea wilt incidence in silty loam soil (85.70%) followed by sandy loam soil (84.70%) and clay soil (78.50%). Soil, on the basis of different particle size distribution, pH, cation exchange capacity, or organic matter content, thus can affect microbial community structure either directly, i.e. by providing a specific habitat that selects specific microorganisms, or indirectly, i.e. by affecting plant root functioning and exudation in a soil-specific manner (Garbeva et al., 2004). The mechanisms by which soils are suppressive to different pathogens, although not always clear, can involve biotic (soil microflora) and/or abiotic factors (soil physicochemical properties); they may vary with the pathogen. Fluctuations in soil moisture content might confound the principles and the relationships between the plant and soil types may influence microbial community (Garbeva et al., 2004). The differences observed in different studies may be due to different crops/different pathogens used.

Proline is a multi-functional amino acid which confers tolerance to plants against abiotic stresses (Szabados and Savoure, 2010) and has been correlated to plant defense against pathogens (Cecchini et al., 2011; Senthil-Kumar and Mysore, 2012). Plant accumulates proline by increasing its synthesis and reducing catabolism under abiotic stresses (Kishor et al., 2005; Verbruggen and Hermans, 2008). Proline content was increased with the increase in the sand concentration in soil. We have found that pathogen inoculation also increased proline contents, possibly due to plant defense against pathogen (Fabro et al., 2004; Verslues and Sharma, 2010). Studies have shown that proline catabolism is enhanced during early stages of plant defense against invading pathogens (Cecchini et al., 2011). Increase in proline contents in carrots grown in different types of soil and after inoculation of pathogens is probably both due to abiotic and biotic stress.

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