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Interactive effect of *Meloidogyne incognita* and *Macrophomina phaseolina* on the development of root–rot disease complex in relation to growth and physiological attributes of chickpea

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Summary The interactive relationship between the root–knot nematode *Meloidogyne incognita* and the root-rot fungus *Macrophomina phaseolina* in a root–rot disease complex of chickpea (*Cicer arietinum* var. *avrodhi*) was studied in a net house. The present study was carried out in such a manner so that the pathogenic potential of *M. incognita* and *M. phaseolina* individually, simultaneously and sequentially could be monitored. The pathogens singly as well as in combination led to significant reduction in growth, yield, nutrient and biochemical parameters. Gaseous exchange parameters like photosynthetic rate, transpiration rate and stomatal conductance were also reduced following infection of plants by the pathogens. However, maximum reduction was noticed in simultaneous inoculation with both pathogens. Sequential inoculation, where *M. incognita* preceded *M. phaseolina* by 15 days, was more damaging to the crop in comparison to that where *M. phaseolina* preceded *M. incognita* inoculation by 15 days. Infection by *M. phaseolina* caused a considerable reduction in the number of galls, egg–masses and nematode multiplication, with the highest reduction observed in plants simultaneously inoculated with the pathogens. Those plants also showed the highest disease severity in terms of percent root–rot. Thus, a manifold action plan to reduce the impact of the root-rot disease complex on chickpea crops has to be formulated.

Additional keywords: Cicer arietinum, gaseous exchange, interaction, nutrients, pathogenic potential

Introduction

Chickpea (*Cicer arietinum* L.) is the second most essential pulse crop after beans in the world both area wise (13.5 million ha) and production (13.1 million tons) (FAOSTAT, 2016). India is the largest producer of chickpea in the world contributing about 63% of the total production. Chickpea generally known as "Chana"/ "Gram" or "Bengal Gram" and widely appreciated as healthy food, is an essential legume having a broad variety of potential nutritional advantages due to its chemical composition (Aliu *et al*., 2016). In addition, it is important mainly for the developing countries, where people are mainly vegetarians and cannot afford the animal proteins for fulfilling their nutritional requirements. Despite India being the largest producer and processor of chickpea in the world, the country also imports large amounts of this pulse annually in order to meet its ever-increasing consumption requirements.

Chickpea production in India has suffered in the last few years due to various constraints that include both biotic and abiotic stresses. Among these constraints, fungal and nematode attacks are considered as the major biotic factors causing significant yield losses in the crop. *Meloidogyne incognita,* one of the most damaging root–knot nematodes, causes significant losses on chickpea. Parasitism by *M. incognita* is characterised by the formation of root galls and deformation of the vascular system of the plant due to formation of giant cells and transfer of nutrients to these cells for use by the nematodes (Palomares–Rius, 2011; Sumbul *et al*., 2015). *Macrophomina phaseolina*, the causal agent of charcoal rot of chickpea, is an important pathogen causing considerable yield losses (Ashraf *et al*., 2005). The fungus is regularly reported from temperate

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and tropical areas of the world, including India, where it is most commonly associated with chickpea (Srivastava *et al*., 2001; Kumar *et al*., 2007; Naseri *et al*., 2018). Infection by *M. phaseolina* results in the formation of red to brown lesions on the roots and stems due to the presence of dark coloured mycelia and black microsclerotia. Eventually the plants become defoliated and wilted (Iqbal and Mukhtar, 2014). Interactive association between *M. incognita* and *M. phaseolina* results in a root–rot disease complex on chickpea that causes more serious yield losses as compared to their individual action (Siddiqui and Husain, 1991; 1992). A plethora of studies has been performed on interaction of nematode–fungus complex, yet there are limited reports on the interactive effects of *M. incognita* and *M. phaseolina* on chickpea.

The present study was performed to monitor the interactive effect of *M. incognita* and *M. phaseolina* on chickpea taking into account different times of inoculation with the two pathogens (individual, concomitant and sequential inoculation) and the associated alterations in growth, yield, physiology, nutrients, and pathogen-related parameters.

Materials and Methods

Preparation and sterilization of soil mixture

Sandy loam soil obtained from a field of the Aligarh Muslim University (AMU), India, was sieved using a 10-mesh sieve. The soil was subsequently mixed with river sand and organic manure in the ratio of 3:1:1. Clay pots (15 cm in diameter) were filled with the soil mixture (1kg/pot). Small amount of water was added to each pot to wet the soil prior to the steam sterilization of the pots at 1.36 atm pressure for 30 minutes. The pot study was conducted in a net house of the Department of Botany, AMU, Aligarh (27°.52'N latitude, 78°.51'E longitude) during the winter season (October to January).

Growth and maintenance of test plants

The chickpea seeds (var. Avrodhi) were surface sterilized by dipping in 0.01% $HgCl_2$ for 2 min, followed by washing twice with distilled water. Prior to sowing, all seeds were treated with charcoal-based commercial culture of *Rhizobium*, chickpea strain. Five chickpea seeds were sown in each pot and the emerged seedlings were thinned to one seedling/pot. Watering of the pots was done as per requirement.

Inoculum preparation of root–knot nematode *Meloidogyne incognita*

Egg masses of *M. incognita* were hand-picked using sterilized forceps from heavily infested roots of eggplant (*Solanum melongena*) on which pure culture of the nematode was maintained. The egg masses were rinsed with distilled water and placed in a coarse sieve (16 mesh size, 10 cm in diameter) covered with crossed double layers of tissue paper and placed in Petri plates containing water just deep enough to contact the egg masses. The Petri plates were incubated at 25°C in the dark. After three days, most of the eggs hatched and the second stage juveniles (J_2) were collected by washing the Petri plates with distilled water.

The water containing hatched J_2 of *M. incognita* was thoroughly agitated for dispensing the nematodes homogenously in the suspension. The number of J_2 in the suspension was counted under the stereoscope. Five counts were made to calculate the average number of J_2/mL in the suspension of each sample and the final concentration was adjusted to 200±5 J_2/mL. Each plant was inoculated with 10 mL of the suspension containing 2000 freshly hatched J_2.

Mass culture of the root–rot fungus *Macrophomina phaseolina*

Macrophomina phaseolina inoculum was obtained from the roots of naturally infected chickpea plants collected from fields in Aligarh district. The fungal culture was purified and maintained on Potato Dextrose Agar medium. Koch's postulates were applied to assure the pathogenicity of *M. phaseolina* on chickpea plants. Large amount of fungal inoculum (mycelium and spores) was

obtained by mass culturing a *M. phaseolina* isolate in Richard's medium (Riker and Riker, 1936) for 15 days at 25°C. The mycelium and spores were subsequently placed on blotting sheets to remove excess water and nutrients. The final inoculum consisting of a mixture of 100 gr macerated wet mycelium and spores was added to 1 L of distilled water. Ten mL of the inoculum was used for the inoculation of each experimental plant.

Inoculation techniques

For the inoculation of plants with *M. incognita* and/or *M. phaseolina* the soil around the roots of one-week-old healthy chickpea seedlings was removed without causing any injury to the root system. Ten mL of inoculum suspension of *M. incognita* and/or *M. phaseolina* was poured around the roots, which were immediately covered with soil. An equal volume of distilled water was added to control plants.

Experimental Design

The experiment was carried out during the winter season in a completely randomized block design with the following variables:

(1) Uninoculated control
(2) *M. incognita* alone
(3) *M. phaseolina* alone
(4) *M. incognita* + *M. phaseolina* simultaneously
(5) *M. incognita* 15 days prior to *M. phaseolina*
(6) *M. phaseolina* 15 days prior to *M. incognita*

Five replicate pots were used for each treatment.

Measurement of plant growth parameters

The plants were harvested four months after emergence and washed gently under tap water to remove the adhering soil particles. Washed plants were labeled according to the treatments. Number of pods per plant and number of nodules per root system were counted visually. Plant height was measured with a measuring tape. Before estimating the plant fresh weight with a physical balance, the excess of water was removed from the plants with blotting paper. For the determination of dry weight, the plants were air-dried in an oven at 60°C for 24 - 48 h before weighing.

Leaf biochemical analysis

Nitrate Reductase Activity (NRA) in leaves was measured by the process of Jaworski (1971). The nitrogen (N) content of the shoot was determined by the method of Lindner (1944), whereas phosphorus (P) and potassium (K) contents were determined by the method of Fiske and Subbarow (1925) and flame photometer, respectively. Chlorophyll and carotenoid contents of leaves were determined by the method of Hiscox and Israelstam (1979) using dimethyl sulphoxide (DMSO).

Recording of gas exchange parameters of chickpea leaves

Gas exchange parameters, such as photosynthesis rate (Pn), transpiration rate (E) and stomatal conductance (Sc), were measured in fully expanded uppermost leaves of plants with an Infra-Red Gas Analyser (IRGA, CID–340, Photosynthesis System, Bio Science, USA). The measurements were carried out on a sunny day at 11 a.m–12 p.m.

Estimation of nematode reproduction in inoculated pots

Number of galls per root system was counted visually. For estimating the number of egg-masses per root system, the method of Daykin and Hussey (1985) was followed. In order to determine the nematode population in soil, 1 kg of soil from each sample was processed by Cobb's sieving and decanting method, followed by Baermann funnel extraction technique (Southey, 1986). The reproductive potential of *M. incognita* in terms of reproduction factor (Rf) was calculated by dividing the final nematode population in soil by the nematode population used for inoculating the plants (Windham and Williams, 1987).

Observations on percent root–rot in inoculated plants

To estimate the disease severity in terms of percent root–rot caused by *M. phaseolina* in chickpea, roots of each plant were initially cut into 5 cm pieces. The pieces were mixed together, and 15 pieces were randomly selected from the mixture. Each root piece was observed visually, and the length of the rotted portion was measured. The percentage of root–rot was estimated by using the following formula:

$$\text{Root-rot}(\%) = \frac{\text{length of rotted portion on root pieces}}{\text{total length of root pieces}} \times 100$$

Root rot index was determined according to four categories: 0 = none; 1 = less than 25%; 2 = 26–50%; 3 = 51–75%; 4 = 76 = 100% (Aoyagi *et al*., 1998). Disease severity was calculated according to the following formula (Aoyagi *et al.*, 1998):

$$\text{Disease severity}(\%) = \Sigma \frac{\text{Disease index x No of plants in each category of index}}{\text{Higher value of the index x No of all inoculated plants}} \times 100$$

Statistical Analysis

All the data were subjected to analysis of variance (ANOVA). Least significant differences (LSD) were calculated at $P \leq 0.05$ using R software, version 2.14.0. Duncan's Multiple Range Test (DMRT) was deployed to denote significant differences between treatments.

Results

Effect of interaction on growth and yield parameters of chickpea

The highest growth parameters were observed in control plants. Both pathogens applied individually or in combination caused significant reduction in plant growth parameters, such as plant height and fresh as well as dry weights, compared to the control (Table 1). However, the highest reduction in plant growth was observed in plants inoculated simultaneously with the pathogens followed by those where nematode preceded the fungal inoculation by 15 days and those where the fungal preceded the nematode inoculation by 15 days. *Meloidogyne incognita* caused a higher reduction in plant growth as compared to that by *M. phaseolina*. Also, the statistical analysis of data showed that the reduction in all the growth parameters of plants inoculated simultaneously with the pathogens did not differ significantly from that of plants inoculated with *M. incognita* 15 days prior to *M. phaseolina*. Likewise, the highest reduction in the number of pods/plant was observed on plants treated with *M. incognita* + *M. phaseolina* and the lowest on plants inoculated with *M. phaseolina* alone (Table 1). A similar trend of reduction was observed in the number of nodules/root system.

Effect of interaction on biochemicals and nutrients of chickpea leaves

All the treatments, either individual or combined, caused significant reduction in the physiological and biochemical parameters of chickpea plants when compared to control plants. Biochemical and nutrients parameters, such as NRA, chlorophyll, carotenoids, N, P and K contents of the chickpea plants showed higher reductions in case of *M. incognita* + *M. phaseolina* inoculated plants compared to control plants. These reductions were not significant statistically when compared to those on plants inoculated with *M. incognita* 15 days prior to *M. phaseolina* inoculation (Table 2).

Effect of interaction on gaseous exchange rate of chickpea

The highest photosynthetic rate (Pn) was recorded in control plants while inoculation of plants with the pathogens, individually and in any combination, reduced photosynthetic rate significantly (Table 3). Maximum reduction in Pn was observed in plants treated with *M. incognita* + *M. phaseolina* followed by those where the nematode preceded the fungal inoculation by 15 days, those where the fungal preceded the nematode inoculation by 15 days, and those inoculated with *M. incognita* alone and *M. phaseolina* alone. Likewise, E and Sc exhibited the same trend of reduction as compared to control plants (Table 3).

Table 1. Effects of *Meloidogyne incognita* and *Macrophomina phaseolina,* singly and combined, on growth, yield and nodulation of chickpea plants (*Cicer arietinum* var. *avrodhi*).

Treatments	Plant height (cm)	Plant weight (g)		Number of pods/plant	Number of nodules/root system
		Fresh	Dry		
Uninoculated control	57.04*±2.29a	39.50±1.46a	7.12±0.31a	21.00±0.76a	43.00±0.71a
M. phaseolina alone	48.40±1.62b	32.71±1.45b	5.68±0.32b	17.60±0.43b	36.60±0.71b
M. incognita alone	44.94±1.30b	30.29±1.27b	5.24±0.27b	16.40±0.52bc	34.40±0.77bc
M. incognita+M. phaseolina	30.68±0.99d	20.48±1.18d	3.49±0.26d	11.00±0.32d	23.20±0.71d
M. incognita→M. phaseolina	34.40±1.16d	23.02±1.37d	3.94±0.24d	12.60±0.69d	26.20±0.63d
*M. phaseolina→M. incogni*ta	39.56±0.89c	27.71±1.17c	4.78±0.31c	15.00±0.45c	31.40±0.45c

+ = simultaneous inoculation with both pathogens, → = nematode or fungal inoculation preceded by 15 days the fungal or the nematode inoculation, respectively. *Values are means of five replicates. Means in each column followed by the same letter(s) do not differ significantly at $P \leq 0.05$.

Table 2. Effects of *Meloidogyne incognita* and *Macrophomina phaseolina,* singly and combined, on nitrate reductase activity (NRA), nitrogen (N), phosphorus (P) and potassium (K) contents of chickpea plants (*Cicer arietinum* var. *avrodhi*).

Treatments	NRA (µmol NO2/g/h)	Fresh leaf content (mg/g)		
		N	P	K
Uninoculated control	0.397*±0.006a	3.220±0.065a	0.310±0.006a	1.570±0.036a
M. phaseolina alone	0.353±0.008b	2.896±0.055b	0.282±0.006b	1.458±0.031b
M. incognita alone	0.330±0.008bc	2.727±0.058bc	0.266±0.007bc	1.364±0.029bc
M. incognita+M. phaseolina	0.280±0.006d	2.297±0.048d	0.229±0.006e	1.189±0.029e
*M. incognita→M. phaseolin*a	0.296±0.006d	2.428±0.030d	0.241±0.006de	1.228±0.028de
M. phaseolina→M. incognita	0.322±0.008c	2.645±0.055c	0.259±0.007cd	1.317±0.030cd

+ = simultaneous inoculation with both pathogens, → = nematode or fungal inoculation preceded by15 days the fungal or the nematode inoculation, respectively. *Values are means of five replicates. Means in each column followed by the same letter(s) do not differ significantly at $P \leq 0.05$.

Table 3. Effects of *Meloidogyne incognita* and *Macrophomina phaseolina,* singly and combined, on chlorophyll, carotenoid, photosynthesis rate (Pn), transpiration rate (E) and stomatal conductance (Sc) of chickpea plants (*Cicer arietinum* var. *avrodhi*).

Treatments	Fresh leaf content (mg/g)		Pn (µmol/ m2/sec)	E (nmol/ m2/sec)	Sc (nmolH2O/ m2/sec)
	Chlorophyll	Carotenoids			
Uninoculated control	2.140*±0.036a	0.142±0.001a	9.284±0.009a	1.711±0.006a	280.236±0.018a
M. phaseolina alone	1.876±0.035b	0.126±0.002b	7.441±0.011b	1.483±0.005b	244.114±0.016b
M. incognita alone	1.756±0.017bc	0.119±0.001bc	7.127±0.008bc	1.376±0.005bc	233.773±0.025bc
M. incognita+M. phaseolina	1.458±0.023d	0.099±0.001d	5.083±0.008d	1.057±0.003d	191.457±0.025e
M. incognita→M. phaseolina	1.548±0.022cd	0.105±0.001d	5.343±0.007d	1.152±0.006d	202.190±0.021de
M. phaseolina→M. incognita	1.675±0.023c	0.115±0.002c	6.655±0.007c	1.331±0.003c	220.686±0.019cd

+ = simultaneous inoculation of both pathogens, → = nematode or fungal inoculation preceded by 15 days the fungal or the nematode inoculation, respectively. *Values are means of five replicates. Means in each column followed by same letter(s) do not differ significantly at $P \leq 0.05$.

Effect of interaction on nematode and fungal multiplication related parameters on chickpea

The greatest Rf, number of galls and egg–masses/root system were recorded in plants inoculated with *M. incognita* alone (Table 4). The multiplication of *M. incognita* and the number of galls/root system in chickpea plants were significantly hampered in the presence of *M. phaseolina* as compared to plants inoculated with *M. incognita* alone. The greatest reduction was observed in plants inoculated with *M. phaseolina* 15 days prior to *M. incognita* inoculation, followed by those inoculated simultaneously with *M. incognita* and *M. phaseolina* and those where *M. incognita* preceded *M. phaseolina* inoculation by 15 days. Similar trend of reduction was recorded in case of the final population of *M. incognita* recovered from the soil of the treated pots. On the other hand, the highest disease severity was observed in *M. incognita* + *M. phaseolina* inoculated plants followed by plants inoculated with the nematode 15 days prior to fungal inoculation, by plants where the fungal preceded the nematode inoculation by 15 days, and by plants inoculated only with *M. phaseolina*. Similarly, the highest root–rot index was recorded in *M. incognita* + *M. phaseolina* inoculated plants, followed by those inoculated with *M. incognita* 15 days prior to *M. phaseolina,* by plants where the fungal inoculation preceded the nematode inoculation by 15 days and by plants inoculated only with *M. phaseolina* (Table 4).

Discussion

It is evident from the present study that the highest and most significant decrease in growth and yield parameters was observed in chickpea plants inoculated simultaneously with *M. incognita* and *M. phaseolina,* which shows a synergistic effect between the fungus and the nematode (Singh *et al*. 2010; Ganaie and Khan, 2011; Ahmed *et al*., 2014). Simultaneous inoculation of plants with the pathogens significantly damaged the roots and root hairs leading to low capacity of the plants to absorb water and nutrients from the soil. The lack of water and nutrients in the plants resulted in poor growth in terms of reduced plant height, fresh and dry weights (Ansari and Mahmood, 2017). The reduction in growth and yield observed in plants inoculated with *M. phaseolina* 15 days prior to *M. incognita* was equal to that in nematode inoculated plants although the fungus had enough time to colonize the roots and make them less suitable for the penetration by the nematode (Meena *et al*., 2016). It is also possible that the toxic metabolites produced by *M. phaseolina* may have destroyed the giant cells which are necessary for the nematode

Table 4. Effects of *Meloidogyne incognita* and *Macrophomina phaseolina,* singly and combined, on disease development in chickpea plants (*Cicer arietinum* var. *avrodhi*).

Treatments	Number of galls/root system	Number of egg-masses/ root system	Number of nematode juveniles/kg soil	Reproduction factor (Rf)	Disease severity (percent root-rot)	Root-rot disease index (0-4)
Uninoculated control	0.00*±0.00e	0.00±0.00e	0.00±0.00e	0.00	0.00±0.00e	0
M. phaseolina alone	0.00±0.00e	0.00±0.00e	0.00±0.00e	0.00	21.23±1.45d	1
M. incognita alone	114.40±4.17a	107.20±2.17a	19659±321.89a	9.82	0.00±0.00e	0
M. incognita+*M. phaseolina*	79.20±3.40c	61.00±0.98c	12771±155.05c	6.38	64.21±2.55a	3
M. incognita→*M. phaseolina*	93.20±4.73b	83.60±1.50b	14416±222.99b	7.20	59.14±2.70b	3
M. phaseolina→*M. incognita*	69.40±2.84d	53.00±2.97d	11605±248.16d	5.80	46.41±1.73c	2

+ = simultaneous inoculation of both pathogens, → = nematode or fungal inoculation preceded by 15 days the fungal or the nematode inoculation, respectively. *Values are means of five replicates. Means in each column followed by same letter(s) do not differ significantly at $P \leq 0.05$.

feeding and reproduction (Ogaraku, 2008; Ahmed *et al*., 2014).

The significant damage to the root nodules observed in plants inoculated with the pathogens, either individually or simultaneously may be due to the heavy galling resulting from *M. incognita* infection, destruction of root tissue by the rotting caused by *M. phaseolina* and/or the inhibitory effects of *M. incognita* and *M. phaseolina* generated toxic metabolites on *Rhizobium* (Hussain and Siddiqui, 1991; 1992). Plants with lower number of nodules were able to fix lesser nitrogen into nitrate, depriving the plants with suitable substrate for the nitrate reductase enzyme. The decrease in NRA in inoculated plants indicates adverse effect of *M. incognita* and *M. phaseolina* on protein synthesis (Naik *et al*., 1982). This decrease also resulted in reduced growth and yield of chickpea plants. Chlorophyll and nutrient (N, P and K) contents of plants also decreased with the highest reduction observed in plants inoculated simultaneously with the pathogens. Plants inoculated simultaneously with both pathogens showed extremely damaged roots with hampered translocation of water and nutrients from roots to the upper parts (Ansari and Mahmood, 2017). Also, the root–knot nematode directs nutrient contents towards the infected giant cells for their own feeding and reproduction, thus depriving the upper parts of the plants from proper nutrient content levels (Sumbul and Mahmood, 2017).

Gaseous exchange parameters, Pn, E and Sc, were highly reduced in *M. incognita* + *M. phaseolina* inoculated plants which may be due to severe infection of the roots resulting in hampered water absorption and nutrient translocation acropetally (Lorenzini *et al*., 1997; Saeed *et al*., 1999; Strajnar, 2012). Ghazalbash and Abdollahi (2012) reported a decrease in gaseous exchange parameters in tomato plants infected simultaneously with *Meloidogyne javanica* and *Fusarium oxysporum* f. sp. *lycopersici*. The authors assumed that stomatal closure reduces the intercellular CO_2 concentration, which might be the cause behind the reduced net photosynthesis.

The reduced number of galls and egg–masses per root system in the presence of *M. phaseolina* indicates that this fungus is deleterious for the multiplication of *M. incognita*. The detrimental effect of *M. phaseolina* on *M. incognita* multiplication may be due to the destruction of root tissues which become unable to support a large number of galls thus affecting *M. incognita* reproduction (Back *et al*., 2002; Al–Hazmi and Al–Nadri, 2015; Meena *et al*., 2016). Decrease in feeding sites impaired nutrient supply to nematode (Hasan 1993; Fazal *et al*., 1998). Moreover, the toxic substances produced by the fungus resulted in the destruction of the giant cells induced by the nematode, as well as in reduction in hatching and immobilization of J_2 (France and Abawi, 1994; Mokbel *et al*., 2007). Plants inoculated with *M. incognita* 15 days prior to *M. phaseolina* produced higher number of egg–masses, galls and nematode population as compared to those inoculated simultaneously with both pathogens (Ogaraku, 2008).

The lowest disease severity was recorded in plants inoculated with *M. phaseolina* alone. Our results are in conformity with those of Senthamarai (2006), Ganaie and Khan (2011) and Ahmed *et al*. (2014). The low disease severity indicates that *M. phaseolina* could not infect the host in the absence of the predisposing factor, i.e. *M. incognita* in this case (Siddiqui and Hussain, 1991; Lobna *et al*., 2016). The highest rotting of chickpea roots was observed when plants were inoculated simultaneously with *M. incognita* and *M. phaseolina*. This may be because both pathogens had equal opportunities to infect the plants, but the presence of the nematode further enhanced the susceptibility of roots to fungal infection (Ganaie and Khan, 2011; Ahmed *et al*., 2014).

The root–rot fungus has an inherent mechanism to get entry into the root and cause root-rot disease. However, in the case of root–rot disease complex, nematode plays a crucial role in assisting the fungus in its pathogenesis and enhancing host susceptibility (Khan, 1984). Wounds caused by

the nematode on plant roots provide entry points for the fungus to infect the roots more rigorously (Inagaki and Powell, 1969). Apart from the wounds, nematodes also lead to different forms of damage to plant roots like split root galls, cracks and crevices due to emergence of swollen females etc. thus allowing the fungus to infect the host root (Evan and Haylock, 1993, Back *et al*., 2002). In addition to morphological disruptions, alterations in the physiological and nutrient status of the root cells infected by the nematode may also be responsible for the appearance of the root-rot disease complex. Giant cells produced by the root–knot nematode are the regions of high metabolic activity (Jones, 1981). These physiological alterations lead to better nutrients availability to the invading fungus and serve as the key factor in establishing the nematode–fungus disease complex (Khan and Muller, 1982; Khan, 1987; Abdel–Momen and Starr, 1998; Castillo *et al*., 1998). Plant root exudates play a key role in attracting both nematode and fungal pathogens (Grayston, 1997; Clarke and Henessy, 1987). Therefore, the root–knot nematode might have altered the root exudates either quantitatively or qualitatively, making them more favourable for the growth of the fungus (Bergeson, 1972; Golden and Van Gundy, 1975; Reddy 1980).

Conclusions

It can be inferred from the present study that the presence of *M. incognita* increased the severity of the root–rot disease caused by *M. phaseolina* in chickpea plants. The interaction between *M. incognita* and *M. phaseolina* even modified the biochemical composition in the plants to assist the growth and multiplication of the pathogens. Therefore, inoculation of plants with both pathogens (either simultaneous or sequential) caused higher damage compared to individual inoculations. However, the greatest damage was observed in plants inoculated simultaneously with the pathogens. When present together, the pathogens caused significant reduction in chickpea growth and yield and modified physiological and biochemical components of the plants to support their growth accordingly. Moreover, *M. incognita* proved to act as a predisposing factor for the infection of plants by *M. phaseolina*. Thus, the interaction between *M. incognita* and *M. phaseolina* should be taken into consideration for the development of strategies for the effective management of the root-rot disease complex in chickpea crops.

Both authors declare that they do not have any conflict of interest.

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Αλληλεπίδραση μεταξύ του κομβονηματώδη *Meloidogyne incognita* και του μύκητα *Macrophomina phaseolina* στην εμφάνιση του συμπλόκου της ασθένειας "σήψη των ριζών" σε σχέση με την ανάπτυξη και τα φυσιολογικά χαρακτηριστικά των φυτών ρεβιθιού

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Περίληψη Η εργασία αφορά στη μελέτη της αλληλεπίδρασης μεταξύ του κομβονηματώδους *Meloidogyne incognita* και του φυτοπαθογόνου μύκητα *Macrophomina phaseolina* στο σύμπλοκο της ασθένειας "σήψη των ριζών" του ρεβιθιού (*Cicer arietinum* var. avrodhi). Η παθογόνος δύναμη των *M. incognita* και *M. phaseolina* μελετήθηκε μεμονωμένα, ταυτόχρονα και διαδοχικά. Τα παθογόνα τόσο ξεχωριστά όσο και σε συνδυασμό προκάλεσαν σημαντική μείωση της ανάπτυξης, της παραγωγής και των θρεπτικών και βιοχημικών παραμέτρων των φυτών ρεβιθιού. Οι παράμετροι ανταλλαγής αερίων, όπως ο ρυθμός φωτοσύνθεσης, ο ρυθμός διαπνοής και η αγωγιμότητα των στοματίων μειώθηκαν επίσης μετά τη μόλυνση των φυτών από τα παθογόνα. Εντούτοις, η μέγιστη μείωση των παραπάνω παραμέτρων διαπιστώθηκε μετά από ταυτόχρονη μόλυνση των φυτών με τα παθογόνα. Η διαδοχική μόλυνση των φυτών, όπου ο νηματώδης *M. incognita* προηγήθηκε του μύκητα *M. phaseolina* κατά 15 ημέρες, ήταν περισσότερο επιβλαβής για την καλλιέργεια σε σύγκριση με εκείνη όπου ο *M. phaseolina* προηγήθηκε του *M. incognita* κατά 15 ημέρες. Η μόλυνση των φυτών από το μύκητα *M. phaseolina* προκάλεσε σημαντική μείωση στον αριθμό των όγκων των μαζών ωών και στον πολλαπλασιασμό του νηματώδους, με τη μέγιστη μείωση να παρατηρείται στα φυτά που μολύνθηκαν ταυτόχρονα με τα παθογόνα. Αυτά τα φυτά εμφάνισαν επίσης τη μεγαλύτερη ένταση της ασθένειας. Τα αποτελέσματα της παρούσας μελέτης έδειξαν ότι για την μείωση των επιπτώσεων του συμπλόκου της ασθένειας "σήψη των ριζών" στην καλλιέργεια του ρεβιθιού είναι απαραίτητη η διαμόρφωση μιας στρατηγικής πολλαπλών μέτρων διαχείρισης.

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