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Interaction between *Meloidogyne incognita* race 3, *Macrophomina phaseolina* and *Bradyrhizobium* sp. in the root-rot disease complex of chickpea, *Cicer arietinum*

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Summary — The effect of *Meloidogyne incognita* race 3, *Macrophomina phaseolina* and *Bradyrhizobium* sp. on root-rot disease complex of chickpea *(Cicer arietinum)* were examined. *M. incognita* and *M. phaseolina* caused statistically equal damage to plant growth when inoculated singly but two pathogens together caused more damage than the sum of total damage caused by both pathogens individually. Inoculation of *Bradyrhizobium* 10 days prior to pathogens resulted in reduced damage. Inoculation of pathogens prior to *Bradyrhizobium* resulted in more damage than prior or simultaneous inoculation of *Bradyrhizobium*. Both *M. phaseolina* and *Bradyrhizobium*, had an adverse effect on nematode multiplication and galling. Both pathogens also had an adverse effect on nodulation.

Résumé — ***Interaction entre* Meloidogyne incognita *race 3*, Macrophomina phaseolina *et* Bradyzhizobium *sp. dans la maladie racinaire complexe du pois chiche*, Cicer arietinum** — Le rôle de *Meloidogyne incognita* race 3, *Macrophomina phaseolina* et *Bradyrhizobium* sp. dans la maladie racinaire complexe du pois chiche, *Cicer arietinum*, a été étudié. *Meloidogyne incognita* et *Macrophomina phaseolina* provoquent des dégâts statistiquement équivalents sur la croissance de la plante lorsqu'ils sont inoculés seuls, mais les dégâts causés par une inoculation simultanée sont supérieurs à la somme des dégâts causés individuellement. L'inoculation de *Bradyrhizobium* 10 jours avant celles des organismes pathogènes diminue les dégâts. De plus, l'inoculation des organismes pathogènes avant celle de *Bradyrhizobium* provoque des dégâts plus élevés que ceux résultant d'une inoculation antérieure ou simultanée de *Bradyrhizobium*. Tant *Macrophomina phaseolina* que *Bradyrhizobium* ont un effet adverse sur la multiplication des nématodes et sur l'indice de galles. Les deux organismes pathogènes ont une action négative sur la nodulation bactérienne.

Key-words : Nematodes, *Meloidogyne, Macrophomina,* fungus interaction, *Bradyrhizobium,* chickpea.

Chickpea, *Cicer arietinum* L., is an important pulse crop of India. This crop is susceptible to root-knot nematode *Meloidogyne incognita* (Kofoid & White) Chitwood and *Macrophomina phaseolina* (Tassi) Goid., which are often associated with roots of chickpea under field conditions. Infected plants are usually stunted and their root systems often show extensive gall development and root-rot symptoms. Moreover, both pathogens had adverse effect on nodulation.

The importance of root-knot nematodes in disease complexes has received much attention. Many reports have indicated that combinations of *Meloidogyne* spp. in root-infecting fungi may cause greater damage than either pathogen alone (Powell & Nusbaum, 1960; Porter & Powell, 1967; Batten & Powell, 1971; Tu & Cheng, 1971; Al-Hazmi, 1985).

In the present investigation the relationship between *Meloidogyne incognita* race 3, *Macrophomina phaseolina* and *Bradyrhizobium* sp. in root-rot disease complex was determined using individual, simultaneous and sequential inoculations.

Materials and methods

Seeds of chickpea cv. P-256 were sown in 15 cm earthen pots containing 1 kg steam sterilized soil. After germination seedlings were thinned to one per pot. One week after germination seedlings were inoculated with nematodes, fungus and *Bradyrhizobium* as shown in Table 1. The second inoculations were made 10 days after the first.

M. incognita was collected from the chickpea field and multiplied on eggplants *(Solanum melongena)* using single egg-mass. *M. incognita* was identified as race 3 using host differential tests (Taylor & Sasser, 1978). Egg-masses were hand picked using sterilized forceps and 2000 freshly hatched juveniles per plant were used as inoculum. For inoculation, the top soil layer around the root was removed carefully so that root was not damaged. The suspension of nematodes was poured around the root and soil was placed as it was earlier.

M. phaseolina was isolated from infected chickpea roots and maintained on potato dextrose agar (PDA).

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Inoculum of this fungus was prepared by culturing the isolate in Richard's (Riker & Riker, 1936) liquid medium for 15 days at 25 °C. Mycelium was collected on blotting sheets and excess of water and nutrients were removed by pressing it between the two folds of the blotting sheets. 100 g mycelium was macerated 1 l distilled water and 10 ml of this suspension containing 1 g fungus was inoculated around the root by removing the top soil layer. After inoculation the soil was replaced as it was earlier.

In most studies *Bradyrhizobium* is used with seeds. In the present study it was used after germination to study the effect of pre- and post-establishment of *Bradyrhizobium* on the disease complex. To prepare the *Bradyrhizobium* inoculum, 100 g commercial bacterial culture of chickpea strain was dissolved in 1 l of distilled water and 10 ml of this which had 1 g inoculum was added around the roots by removing the top soil layer.

In the treatments where *M. phaseolina* was inoculated, reisolation of fungus was made from galled tissues to confirm its infectivity. The galled portions of root were placed in 0.1 % mercuric chloride for 1 min and washed gently with distilled water at least three times. Roots were then placed in PDA for 7 days at 27 °C. The growth of the fungus was found from the galled portion of roots and later identified as *M. phaseolina.*

Each set was replicated three times and watered as needed. The experiment was terminated ninety days after the first inoculation. Data were recorded as dry plant weight, number of galls and nodules, root-rot index and nematode density. The soil nematode population was estimated by Cobb's sieving and decanting technique. The numbers of juveniles, eggs and females in the roots were also estimated. The roots were cut into small pieces and mixed homogeneously. One gram root from this was taken and macerated for 45 s in a blender and counted. A root-rot index was conducted by recording disease severity on a 0 to 5 scale where 0 = no disease and 5 = severe root-rot. Data recorded were analysed statistically using multifactorial analysis and critical differences were calculated at 5 % level.

Results

Inoculation of *Bradyrhizobium* 10 days prior to one or both of the pathogens resulted in significantly less damage to plant growth and nodulation than simultaneous inoculation of *Bradyrhizobium* and pathogens. In contrast, inoculation of plants with one or both the pathogens prior to *Bradyrhizobium* resulted in significantly greater reduction in plant growth and nodulation than the simultaneous inoculation. When pathogens were inoculated without *Bradyrhizobium* the damage to plant growth was higher than with all the other treatments.

Out of three organisms, the treatment of *Bradyrhizobium* alone resulted in improved plant growth as compared to uninoculated control. The two pathogens, i.e. *M. incognita* and *M. phaseolina,* were equally effective in reducing plant growth and nodulation but inoculation of both the pathogens together caused more damage than the sum of damage caused by both pathogens individually. *M. phaseolina* and *Bradyrhizobium* had an adverse effect on nematode multiplication and galling but the effect of *M. phaseolina* was greater than that of *Bradyrhizobium.*

The interaction effect was also significant in terms of plant growth and nematode density. Inoculation of *Bradyrhizobium* alone, before or 10 days later led to equal plant growth. Inoculation of *Bradyrhizobium* 10 days after *M. incognita* or *M. phaseolina* resulted in similar reduction in plant growth as caused by *M. incognita* or *M. phaseolina* without *Bradyrhizobium.* The effect of *Bradyrhizobium* was nullified by the prior establishment of the pathogens.

The plants inoculated with *M. incognita* and *M. phaseolina* singly or simultaneously suffered 33, 28 and 77 % shoot growth damage over uninoculated control. In cases where plants were inoculated with *M. incognita* or *M. phaseolina* or both simultaneously with *Bradyrhizobium* there was 26, 23 and 51 % reduction in shoot growth over plants inoculated with *Bradyrhizobium* alone. When the pathogens were inoculated first followed by *Bradyrhizobium* the damage was 34, 31 and 69 % respectively while the prior inoculation of *Bradyrhizobium* followed by pathogens resulted in only 18, 14 and 41 % reduction in shoot growth.

The reduction in nodulation was found lowest (8 %) when *Bradyrhizobium* was inoculated first followed by *M. phaseolina.* The reduction was highest (58 %) when *M. incognita* and *M. phaseolina* were inoculated together first followed by *Bradyrhizobium.* In other treatments the reduction ranged between 8-58 % (Table 1).

The nematode multiplication was 23 times higher than the initial inoculum when *M. incognita* was inoculated alone. The multiplication was reduced to 19-fold on inoculation of *M. incognita* plus *M. phaseolina.* With *M. incognita* in the presence of *Bradyrhizobium* multiplication increased 20 times over initial inoculum. When *M. incognita, M. phaseolina* and *Bradyrhizobium* were inoculated simultaneously multiplication was 13 times greater. The multiplication was found at its minimum (11 times) when *Bradyrhizobium* was inoculated first followed by *M. phaseolina* and *M. incognita* later (Table 1).

Discussion

Inoculation of *Bradyrhizobium* improved plant growth over uninoculated control by increasing nitrogen status of the soil. In cases where *Bradyrhizobium* was used with pathogens it also improved plant growth by producing antibiotics against pathogens. *Bradyrhi-*

Table 1. Effect of individual, simultaneous and sequential inoculation of *Meloidogyne incognita, Macrophomina phaseolina* and *Bradyrhizobium* sp. on dry weight, nodulation, disease development and nematode multiplication on chickpea.

Treatments	Dry Wt. (g)		No. of nodules	Nematode population (1000's)	No. of galls				
	Shoot	Root							
Path. effect	3.34	1.07		41.9	300				
B. + Path. effect	4.34	1.40	29	32.6	260				
Path. → B. effect	3.84	1.22	26	35.6	273				
B. → Path. effect	4.73	1.52	34	30.0	219				
C. D. 5 %	0.22	0.08	2.2	0.5	17.0				
B. effect	5.60	1.81	39	—	—				
MI effect	4.07	1.30	29	41.5	298				
MP effect	4.27	1.34	30	—	—				
MI + MP effect	2.31	0.75	20	28.5	228				
C. D. 5 %	0.22	0.08	2.5	0.4	12.0				
						% supp. in dry shoot weight	% supp. in nodules	Nematode multiplication	Root-rot index
	Uninoc. Control	5.07	1.62	—	—	—	—	—	—	—
Without	MI	3.42	1.09	—	46.5	327	33	—	23	—
Bradyrhizobium	MP	3.67	1.13	—	—	—	28	—	—	5
	MI + MP	1.18	0.42	—	37.3	272	77	—	19	5
	B	5.77	1.88	39	—	—	—	—	—	—
Simultaneous	MI + B	4.29	1.39	28	40.0	291	26	28	20	—
inoculation	MP + B	4.47	1.42	30	—	—	23	23	—	4
	MI + MP + B	2.83	0.90	19	25.1	228	51	51	13	5
	O → B	5.76	1.86	38	—	—	—	—	—	—
Bradyrhizobium	MI → B	3.82	1.20	24	42.3	316	34	37	21	—
after	MP → B	3.98	1.24	24	—	—	31	37	—	5
pathogens	MI + MP → B	1.79	0.59	16	28.9	229	69	58	14	5
	B → O	5.77	1.88	39	—	—	—	—	—	—
Bradyrhizobium	B → MI	4.76	1.53	34	37.1	256	18	13	19	—
prior to	B → MP	4.95	1.57	36	—	—	14	8	—	3
pathogens	B → MI + MP	3.43	1.08	26	22.8	182	41	33	11	5
C.D. 5 %		0.45	0.17	N.S.	0.7	N.S.				

B = *Bradyrhizobium*, MI = *Meloidogyne incognita*, MP = *Macrophomina phaseolina*, + = Simultaneous inoculation, → = Inoculation 10 days later, 0 = Zero.

zobium has been reported to produce antipathogenic substances (Roslycky, 1967; Schwinghamer & Belkengren, 1968; Marx, 1969; Drapeau *et al.*, 1973) and plants inoculated with *Bradyrhizobium* suffered less damage by pathogens than uninoculated plants (Sharma & Sethi, 1976; Bopaiah *et al.*, 1976; Tu 1978, 1980). Prior establishment of *Bradyrhizobium* produced more antibiotics and improved plant growth more than when *Bradyrhizobium* became established together with or after pathogens. Both pathogens inoculated together caused more damage than did pathogens introduced singly, probably because nematodes predisposed the plants to fungus attack which resulted in more damage (Batten & Powell, 1971; Mani & Sethi, 1987). Nematode and fungus together have a greater negative effect on nodulation than any of them singly (Malek & Jenkins, 1964; Mani & Sethi, 1987).

Adverse effect of *Bradyrhizobium* on nematode multiplication can be attributed to their antibiotics production. Adverse effect of *M. phaseolina* on nematode multiplication as observed in the present study has been observed by others (Al-Hazmi, 1985; Sakhuja & Sethi, 1986). *M. phaseolina* which was isolated from galled roots might have affected nematode feeding, resulting in less nematode multiplication. Powell (1971) was also of the same opinion that population of sedentary nematodes, as a result of interaction with fungi, are reduced due to adverse effect on nematode penetration and direct fungus invasion disrupting nematode feeding and subsequent reproduction within the roots.

Chupp and Sherf (1960) reported *M. phaseolina* as a weak pathogen but in the present study it caused significant damage. The difference in result may be due to different isolate of the fungus used or due to difference in the experimental conditions.

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