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Section of Plant Pathology and Nematology
Department of Botany, Aligarh Muslim University, Aligarh, India

INTERACTIONS OF *MELOIDOGYNE JAVANICA*, *ROTYLENCHULUS RENIFORMIS*, *FUSARIUM OXYSPORUM* F. SP. *CICERI* AND *BRADYRHIZOBIUM JAPONICUM* ON THE WILT DISEASE COMPLEX OF CHICKPEA

by
Z. A. SIDDIQUI and I. MAHMOOD

Summary. The effects of *Meloidogyne javanica*, *Rotylenchulus reniformis*, *Fusarium oxysporum* f. sp. *ciceri* and *Bradyrhizobium japonicum* on the disease complex of chickpea (*Cicer arietinum*) were examined. Individually, *F. oxysporum* reduced chickpea growth more than *M. javanica*, or *R. reniformis* but the inoculation of the pathogens together caused more damage than sum of damage caused by the pathogens singly. The highest suppression in plant growth was caused when *M. javanica* and *R. reniformis* were inoculated 10 days prior to *F. oxysporum*. Plants suffered less damage from pathogens when inoculated with *B. japonicum*. The inoculation of *B. japonicum* prior to the pathogens limited visible damage more so than later inoculation. Prior establishment of one nematode species was antagonistic to the multiplication of the other subsequently inoculated nematode species. Both *B. japonicum* and *F. oxysporum* had adverse effects on nematode multiplication. Greatest suppression in nematode multiplication was caused when *F. oxysporum* was inoculated prior to *M. javanica*, *R. reniformis* and *B. japonicum*. Each of the three pathogens adversely affected nodulation.

Chickpea (*Cicer arietinum* L.) is susceptible to root-knot nematode *Meloidogyne javanica*, *Rotylenchulus reniformis*, and *Fusarium oxysporum*. A complex of these pathogens is often found associated with the roots of chickpea. Infected plants grow poorly, show marked symptoms of wilting and have extensive galling on the roots.

Although the root-rot disease complex of chickpea caused by *Meloidogyne incognita* and *Macrophomina phaseolina* has been studied (Siddiqui and Husain, 1991, 1992) the often associated disease complex with *Fusarium* has not been properly investigated.

In the present work the relationship between *M. javanica* (Treub) Chitw., *R. reniformis* Linford *et* Oliveira, *F. oxysporum* f. sp. *ciceri* (Padw.) Synd. *et* Hans and the nodule producing bacterium *Bradyrhizobium japonicum* Jordan in the wilt disease complex of chickpea were investigated.

Materials and methods

Seeds of chickpea cv. P-256 were sown in 15 cm clay pots containing 1 kg steam sterilized soil. After germination the seedlings were thinned to one per pot. One week after germination they were inoculated with nematodes, fungus and/or *B. japonicum* as shown in Tables (I-IV). The second inoculations were made 10 days after the first.

Meloidogyne javanica was collected from a chickpea field and cultured on egg plants (*Solanum melongena* L.) using a single eggmass per plant. Egg masses were hand picked using sterilized forceps and 2000 freshly hatched juveniles per plant were used as inoculum. For inoculation, the surface soil layer around the root was removed and the suspension of nematodes then poured around the root and the surface soil replaced.

Rotylenchulus reniformis collected from a chickpea field was cultured on castor (*Ricinus cuminis* L.). Soil from around the castor plant roots was processed by Cobb's sieving and decanting technique followed by Baermann funnel. After 24 hrs nematodes were collected and kept in an incubator at 25 °C for a week, with the water changed every 24 hrs, to obtain immature females of *R. reniformis*. Approximately, 2000 immature females of *R. reniformis* per plant were inoculated similarly to *M. javanica*.

Fusarium oxysporum was isolated from infected chickpea roots and maintained on potato dextrose agar (PDA). Inoculum of the fungus was prepared by culturing the isolate in Richard's liquid medium (Riker and Riker, 1936) for 15 days at 25 °C. Mycelium was collected on blotting paper and excess water and nutrients removed by pressing it between two folds of the paper. About 100 g mycelium was comminuted in 1 l of distilled water and 10 ml of the suspension containing 1 g fungus was inoculated around the roots of each plant in a similar manner to the nematode inoculations.

In most studies *B. japonicum* is inoculated on to the seeds when they are sown, but in our study it was applied

TABLE I - *Effect of simultaneous, pre and post inoculation of* Meloidogyne javanica *with* Fusarium oxysporum, Rotylenchulus reniformis *and* Bradyrhizobium japonicum *on the growth of chickpea and development of disease.*

Treatments		Dry shoot weight (g)	No. of nodules per root system	Nematode population		Wilting index
				Root-knot (x 10^3)	Reniform	
	Control	6.1	5	–	–	–
	Mj	4.5	2	45	–	–
	Mj+Rr	2.7	0	27	22	–
Simultaneous	Mj+Fo	2.6	0	35	–	4
inoculation	Mj+Bj	5.0	24	40	–	–
	Mj+Rr+Fo	1.0	0	12	10	5
	Mj+Rr+Bj	3.2	16	15	13	–
	Mj+Fo+Bj	3.0	17	18	–	4
	Mj+Rr+Fo+Bj	1.3	6	8	6	5
	Control	6.2	4	–	–	
	Mj→O	4.5	1	46	–	
M. javanica	Mj→Rr	2.6	0	30	18	–
Inoculated	Mj→Fo	2.4	0	38	–	5
10 days	Mj→Bj	4.9	15	43	–	–
prior	Mj→Rr+Fo	0.9	0	15	9	5
	Mj→Rr+Bj	3.6	12	17	11	–
	Mj→Fo+Bj	2.7	14	20	–	4
	Mj→Rr+Fo+Bj	1.2	4	10	5	5
	Control	6.1	5	–	–	–
	O→Mj	4.6	2	45	–	–
	Rr→Mj	2.9	0	24	24	–
M. javanica	Fo→Mj	2.9	0	33	–	4
Inoculated	Bj→Mj	5.4	25	37	–	
10 days	Rr+Fo→Mj	1.4	0	10	17	5
later	Rr+Bj→Mj	3.4	15	14	13	–
	Fo+Bj→Mj	3.5	22	15	–	3
	Rr+Fo+Bj→Mj	1.5	10	7	7	5
C.D. P=0.05		0.2	2.3	1	1	-

Mj = *Meloidogyne javanica*, Rr = *Rotylenchulus reniformis*
Fo = *Fusarium oxysporum*, Bj = *Bradyrhizobium japonicum*

after germination to determine the effect of pre and post-establishment of *B. japonicum* on the disease complex. To prepare the *B. japonicum* inoculum, 100 g commercial bacterial culture (*Bradyrhizobium japonicum*) of the chickpea strain were suspended in 1 l distilled water and 10 ml of the inoculum suspension were added around the roots by removing the top soil layer.

In the treatments using *F. oxysporum* the fungus was re-isolated from galled tissues to confirm that infection had occurred. Galled portions of roots were surface sterilized in 0.1% mercuric chloride for 1 min., washed gently with distilled water at least three times and then cultured on PDA plates for 7 days at 27 °C. Fungal growth from the galled portion of the roots was later identified as *F. oxysporum* f. sp. *ciceri*.

Each treatment was replicated five times and pots were watered as needed.

The experiment was terminated 90 days after the first inoculation. Data were recorded on dry weight, number of nodules, wilting index and nematode density. Nematode populations of both root-knot and reniform nematodes were recorded separately. Soil nematode populations were extracted by Cobb's sieving and decanting technique and counted. The number of juveniles, eggs and females in the

TABLE II - *Effect of simultaneous, pre and post inoculation of* R. reniformis *with* M. javanica, F. oxysporum *and* B. japonicum *on the growth and disease development of chickpea.*

Treatments		Dry shoot weight (g)	No. of nodules per root system	Nematode population		Wilting index
				Root-knot ($x 10^3$)	Reniform	
Simultaneous inoculation	Control	6.1	4	–		-
	Rr	4.7	2	41	–	-
	Rr+Mj	2.7	0	21	26	–
	Rr+Fo	2.7	0	38	–	4
	Rr+Bj	5.3	27	32	–	–
	Rr+Mj+Fo	**1.1**	0	11	13	5
	Rr+Mj+Bj	3.1	16	13	16	–
	Rr+Fo+Bj	3.3	20	15	-	4
	Rr+Mj+Fo+Bj	1.3	5	26	**8**	5
R. reniformis Inoculated 10 days prior	Control	6.1	5	-	–	-
	Rr→O	4.7	3	41	-	–
	Rr→Mj	2.9	0	26	23	–
	Rr→Fo	2.5	0	35	-	**4**
	Rr→Bj	5.1	21	40	-	–
	Rr→Mj+Fo	1.0	0	13	**11**	**5**
	Rr→Mj+Bj	3.0	15	15	**12**	-
	Rr→Fo+Bj	2.8	18	18		4
	Rr-Mj+Fo+Bj	1.2	**4**	8	6	5
R. reniformis Inoculated 10 days later	Control	6.2	4	–	-	-
	O→Rr	4.8	1	37	-	-
	Mj→Rr	2.6	0	18	30	-
	Fo→Rr	3.0	0	29	-	**4**
	Bj→Rr	5.4	30	35	–	–
	Mj+Fo→Rr	1.2	0	8	15	**5**
	Mj+Bj→Rr	3.3	14	10	17	-
	Fo+Bj→Rr	3.6	24	12	-	**3**
	Mj+Fo+Bj→Rr	1.5	8	4	**10**	**5**
C.D. P=0.05		**0.1**	2.2	**1**	**1**	–

roots were also estimated. For root-knot nematodes, roots were cut into small pieces and mixed homogeneously. One g of root was comminuted in a blender for 4-5 seconds and nematodes were counted with a stereomicroscope. Females of the reniform nematode on the roots were counted by staining the roots and counting the females with the aid of a stereomicroscope. A wilting index recorded disease severity on a 0 to 5 scale where 0=no wilting and 5=severe wilting.

Data were analysed statistically using multifactorial analysis and critical differences were calculated at the 5% level with ANOVA. The experiment was conducted in 1990 and repeated in 1991. The results were similar in both years so only the 1991 data is presented.

Results

Individually, *F. oxysporum* was more pathogenic than *M. javanica* or *R. reniformis* (Tables I, II and III). When the pathogens were inoculated together, the reduction in dry shoot weight was greater than the sum of the damage caused by the pathogens individually. Inoculation of the three pathogens together resulted in greater damage than in any other treatment (Table I). Inoculation of *M. javanica* prior to *R. reniformis*, *F. oxysporum* and *B. japonicum* resulted in greater suppression in dry shoot weight than simultaneous or subsequent inoculation. Dry shoot weight was reduced by a similar amount when *M. javanica* and *R. reniformis* were inoculated simultaneously or when

M. javanica was inoculated in pre or post-inoculations with *R. reniformis*. Inoculation with *M. javanica* prior to *R. reniformis* and *F. oxysporum* reduced dry shoot weight by the same amount as when they were inoculated simultaneously.

Inoculation of *R. reniformis* with *M. javanica* reduced dry shoot weight in a similar amount as was caused by *F. oxysporum* along with *R. reniformis* (Table II). Inoculation with *R. reniformis* prior to the other test organisms resulted in greater damage followed by its simultaneous or subsequent inoculation. Results were similar when either *R. reniformis* was inoculated prior to *M. javanica* and *F. oxysporum* or they were inoculated simultaneously. Prior inoculation of *R. reniformis* to *M. javanica* and *B. japonicum* suppressed dry shoot weight to the same extent as when were they inoculated simultaneously.

Prior inoculation of *F. oxysporum* resulted in less damage compared with simultaneous or later inoculation with test organisms. Simultaneous inoculation of *F. oxysporum* and *M. javanica* was at par when *F. oxysporum* was inoculated prior to *M. javanica*. The greatest loss in dry shoot weight occurred when *F. oxysporum* was inoculated after *M. javanica* and *R. reniformis*, followed by simultaneous and prior inoculation of *F. oxysporum* to both nematode species. Inoculation of *F. oxysporum* prior to *M. javanica* and *B. japonicum* did not differ from simultaneous inoculation. Inoculation of *F. oxysporum* prior to or later than *R. reniformis* and *B. japonicum* resulted in similar dry shoot weight (Table III).

TABLE III - *Effect of simultaneous, pre and post inoculation of* oxysporum *with* M. javanica, R. reniformis *and* B. japonicum *on the growth and disease development of chickpea.*

		Dry shoot weight (g)	No. of nodules per root system	Nematode population		Wilting index
				Root-knot (x 10^3)	Reniform	
Simultaneous inoculation	Control	6.2	5	–	–	–
	Fo	4.3	2	–	–	**3**
	Fo+Mj	2.6	0	35	–	**4**
	Fo+Rr	2.7	0	–	32	**4**
	Fo+Bj	4.7	21	–	–	2
	Fo+Mj+Rr	1.0	0	12	10	5
	Fo+Mj+Bj	3.2	17	17	–	4
	Fo+Rr+Bj	3.3	20	–	15	4
	Fo+Mj+Rr+Bj	1.3	10	8	6	5
F. oxysporum Inoculated 10 days prior	Control	6.2	6	–	–	–
	Fo→O	4.3	2	–	–	3
	Fo→Mj	2.7	0	28	–	4
	Fo→Rr	2.8	0	–	26	4
	Fo→Bj	4.9	17	–	–	2
	Fo→Mj+Rr	1.3	0	10	**7**	**5**
	Fo→Mj+Bj	3.3	20	14	–	4
	Fo→Rr+Bj	3.5	23	–	12	3
	Fo→Mj+Rr+Bj	1.4	12	6	3	5
F. oxysporum Inoculated 10 days later	Control	6.2	4	–	–	–
	O→Fo	4.4	3	–	–	3
	Mj→Fo	2.4	0	37	–	5
	Rr→Fo	2.5	0	–	34	4
	Bj→Fo	5.0	25	–	–	2
	Mj+Rr→Fo	0.8	0	15	11	5
	Mj+Bj→Fo	2.9	16	19	–	**4**
	Rr+Bj→Fo	3.5	18		16	**3**
	Mj+Rr+Bj→Fo	1.3	**7**	10	**7**	**5**
C.D. P=0.05		0.2	2.3	**1**	**1**	–

Inoculation of *B. japonicum* with the pathogens restricted their damaging effect (Table IV). Inoculation of *B. japonicum* and both nematode species had the same effect on dry shoot weight as *B. japonicum* inoculated along with *M. javanica* and *F. oxysporum*. Inoculation of *B. japonicum* after *M. javanica* and *R. reniformis* was at par with their simultaneous inoculation. Similarly, inoculation of *B. japonicum* prior to *M. javanica* and *F. oxysporum* was at par with their simultaneous inoculation (Table IV).

Few nodules were found on plants without *B. japonicum* but no nodule formation was observed when plants without *B. japonicum* were inoculated with two or more pathogens (Table I). Suppression in the number of nodules was more when both the nematodes species were inoculated with *B. japonicum* than plants inoculated along with *M. javanica*, *F. oxysporum* and *B. japonicum*. The greatest suppression in nodulation was observed when all four organisms were inoculated compared with plants inoculated with *B. japonicum* alone. Inoculation of *M. javanica* after the other three organisms increased nodulation compared with simultaneous inoculation, but prior inoculation of *M. javanica* to the other three organisms was at par to their simultaneous inoculation (Table I).

Inoculation of *R. reniformis* prior to other test organisms was at par in terms of nodulation when they were inoculated simultaneously while later inoculation of *R. reniformis* with other test organisms was better than simultaneous or prior inoculation (Table II). Inoculation of B. japonicum after *R. reniformis* resulted in less nodulation compared with their simultaneous inoculation. Nodulation was at par when *R. reniformis* was inoculated simultaneously, prior to or later than *M. javanica* plus *B. japonicum* (Table II).

Pre, post and simultaneous inoculation of *F. oxysporum* with test organisms had a statistically similar effect on nodulation (Table III). Nodulation was suppressed more when *F. oxysporum* was inoculated prior *B. japonicum* followed by simultaneous and later inoculations. Inoculation of *F. oxysporum* prior to *M. javanica* and *B. japonicum* was better for nodulation as compared to their simultaneous inoculation.

Inoculation of *B. japonicum* with *M. javanica* exerted a similar effect on nodulation as that caused by *B. japonicum* and *F. oxysporum* inoculation. Inoculation of either of the two test pathogens simultaneously or after *B. japonicum* exerted a similar effect on nodulation (Table IV).

Multiplication of *M. javanica* was greatest when inoculated alone (Table I). The presence of *R. reniformis*, *F. oxysporum* and *B. japonicum* significantly reduced multiplication of *M. javanica* but *R. reniformis* was highly effective in suppressing multiplication of *M. javanica* followed by *F. oxysporum* and *B. japonicum*. In sequential inoculations, prior inoculation of *M. javanica* with the other test organisms was found best for multimlication of *M. javanica* followed by simultaneous and later inoculation. Where both nematode species were inoculated simultaneously, multiplication of *M. javanica* was greater than *R. reniformis*. Prior inoculation of *R. reniformis* with *M. javanica* adversely affected multiplication of the latter (Table II). Prior inoculation of *F. oxysporum* or *B. japonicum* had a considerable adverse effect on multiplication of both nematode species followed by their simultaneous or later inoculations (Table III and IV).

Wilting index was estimated as 3 when *M. javanica* was inoculated after *F. oxysporum* along with *B. japonicum*. The index was 4 when *M. javanica* was inoculated prior or simultaneously with *F. oxysporum* and *B. japonicum* (Table I). Simultaneous inoculation of *M. javanica* and *F. oxysporum* or inoculation of *M. javanica* after *F. oxysporum* resulted in a wilting index 4. Other treatments of *F. oxysporum* with test organisms produced a wilting index 5 (Table I). Inoculation of *F. oxysporum* and *B. japonicum* prior to *R. reniformis* resulted in a wilting index 3. In other treatments with *F. oxysporum* the indices were 4-5 (Table II). Inoculation of *B. japonicum* with *F. oxysporum* produced the lowest wilting index 2 (Table III). Inoculation of *F. oxysporum* alone produced an index 3, the same as when *F. oxysporum* was inoculated prior to or later than *R. reniformis* and *B. japonicum*. In other treatments the indices were 4-5 (Table III). Inoculation of *B. japonicum* prior to *R. reniformis* and *F. oxysporum* gave an index 3 (Table IV).

Discussion

Inoculation of nematodes prior to other pathogens caused higher damage than the simultaneous inoculation because of biochemical changes in various tissues of the host brought about by nematodes favouring the growth of the fungus (Batten and Powell, 1971). *Fusarium oxysporum* adversely affected nematode multiplication as reported by Mani and Sethi (1987). Powell (1971) reported that as a result of interaction of nematode and fungi, the population of sedentary nematodes are suppressed due to adverse effects on nematode penetration and direct fungus invasion disrupting nematode feeding and subsequent reproduction within the roots. Suppression of reproduction of both the nematodes in combined inoculations may be due to antagonistic relationship of both nematode species.

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TABLE IV - *Effect of simultaneous, pre and post inoculation of* B. japonicum *with* M. javanica, R. reniformis *and* F. oxysporum *on the growth and disease development of chickpea.*

Treatments		Dry shoot weight (g)	No. of nodules per root system	Nematode population		Wilting index
				Root-knot (x 10^3)	Reniform	
Simultaneous inoculation	Control	6.1	5	–	–	–
	Bj	6.4	37	–	–	–
	Bj+Mj	5.1	24	40	–	–
	Bj+Rr	5.3	27	–	38	–
	Bj+Fo	4.8	25	–	–	2
	Bj+Mj+Rr	3.2	15	15	12	–
	Bj+Mj+Fo	3.1	17	18	–	4
	Bj+Rr+Fo	3.3	18	–	15	4
	Bj+Mj+Rr+Fo	1.3	7	9	6	5
B. japonicum Inoculated 10 days prior	Control	6.2	6	–	–	–
	Bj→O	6.4	39	–	–	–
	Bj→Mj	5.4	28	38	–	–
	Bj→Rr	5.4	33	–	35	–
	Bj→Fo	5.0	30	–	–	2
	Bj→Mj+Rr	3.3	15	13	10	–
	Bj→Mj+Fo	3.3	17	16	–	4
	Bj→Rr+Fo	3.8	20	–	12	3
	Bj→Mj+Rr+Fo	1.6	14	6	5	5
B. japonicum Inoculated 10 days later	Control	6.2	3	–	–	–
	O→Bj	6.4	33	–	–	–
	Mj→Bj	4.9	19	43	–	–
	Rr→Bj	5.1	22	–	40	–
	Fo→Bj	4.9	17	–	–	2
	Mj+Rr→Bj	3.0	3	16	14	–
	Mj+Fo→Bj	2.9	5	20	–	4
	Rr+Fo→Bj	3.1	7	–	16	4
	Mj+Rr+Fo→Bj	1.1	2	11	8	5
C.D. P=0.05		0.2	2.6	1	1	–

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