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Research article

Interaction between the nematodes *Heterodera cajani, Meloidogyne incognita, Rotylenchulus reniformis* and the fungus *Fusarium solani* on cowpea plants

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Abstract

Effects of concomitant inocula of *H. cajani* (Hc)*, M. incognita* (M_2) and *R. reniformis* (Rr) on cowpeas showed that more eggs, L_2 and cysts were developed in cowpea roots in the *H. cajani* alone treatment than in the three nematode pathogen treatments. The final cyst population –being, increased with the increase of inoculated larvae is positively correlated with the larval inoculate level of *H cajani.* With the exception of the treatment 1000Hc+ 4000 Rr, statistical more *H. cajani* L_2 population were extracted from roots of the concomitant nematode treatments than from the *H. cajani* single inoculated treatment.

Key words: Pathogeneses; the nematodes*: H. cajani*, *M incognita*, *R. reniformis*; the fungus *Fusarium solani*; cowpea, N, P, K, Zn, Mn, Fe, Cu

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1. Introduction

The discovered cyst nematode species hitherto detected in Egyptian agricultural soil were found to attack the roots of field and vegetable crops like berseem, corn, wheat, cowpea, snapbeen and other leguminous crops. Sharma and Sethi [1] found that the leghaemoglobin content of cowpea nodules decreased as a result of infestation with *M. incognita* and *H. cajani. M. incognita* causes a reduction of 51.56%, *H. cajani* of 36.65%, while the nematodes in combination caused a reduction of 44.1%. Sharma and Sethi [1] found that populations of each of *M. incognita* and *H. cajani* were maximum when inoculated separately on the cowpea. They found also that increased in final population was density dependent. The host infestation and total multiplication of both species were mutually inhibited by each other. Penetration sites of *M incognita* and *H cajani* differed considerably as *H. cajani* larvae penetrated faster and higher number than *M. incognita.* The durations of the life cycle of either of the species were similar in concomitant and single inoculation. Sharma and Sethi [2] found that *M. incognita* and *H. cajani* significantly reduced plant growth, nodulation and shoot nitrogen content. Both species penetrated and developed in root nodules. *M. incognita* reduced the nitrogen content more than *H. cajani*. Massoud [3] found that plants infected with two species from *H. trifoli (=H.daverti), M. incognita* and *R. reniformis* at the same time showed some growth reduction than those alone with either species. She found that the nematode development and reproduction of a given species was affected by the presence of the other species on the same host. Jatala and Jensen [4,5] found that there was suppression of gall development of *M. hapla* when *H. schachtii* preceded *M. hapla* and this interrelationship was characterized by amensalism. When *M. hapla* infection proceeded *H. schachtii* with 10 days, there was an increase in cyst development and the relationship was characterized by commensalisms. Niblack *et al.* [6] showed that interactions between *H. glycines* and *M. incognita* did not affect early plant growth or number of nematodes penetrating roots. *H. glycines* and *M. incognita* were higher in sand than in heavier soil, and higher in the greenhouse than in the field.

Several reports have been published describing interaction cases between cyst nematodes and certain soil fungi. Polychronopoulos *et al.* [7] found that sugar beet seedlings infected with *H. schachtii* and *Rhizoctonia solani* were killed within 3 days. The fungus alone did not cause such rapid death. They showed that also in the nematode plus fungus attack, the fungus first penetrates the giant cells produced by the nematode and spreads from these into healthy cells. Meagher and. Chambers [8] found that in glasshouse tests on wheat, *H. avenae* and *R. solani* in combination caused a significantly greater reduction in plant height, fresh weight and root number and length than either pathogen individually. Root weight was not significantly affected by nematode and fungus in combination. Meagher *et al.* [9] found that the combination of *H. avenae* and *R. solani* caused more reduction in growth than infestation with either alone. In wheat plants infested with *H. avenae*, emergence was delayed for 10 days and for 13 days by *H. avenae* + *F solani* complex. Gill and Swarup [10] found that the size and weight of shoot and roots of barley plants were significantly depressed by both Helminthosporiuim gramineum and particularly *Fusarium moniliforme* fungi. *H. aveime* reduced root growth at the highest inoculum levels. There was no significant overall interaction on plant growth between the fungi and nematodes. The fungi reduced cyst production when present singly. Nordmeyer and Sikora [11] found that root of seedlings of *Trifolium subterraneum* exposed for 7.5 min to a culture filtrate of *Fusarium avenaceum* were penetrated by juveniles of *H. daverti* to a greater extent than roots not exposed. Dry weight of clover *Trifolium subterraneum* was reduced synergistically when *H. daverti* was inoculated one or two weeks after a *Fusarium oxysporum* fungus. Nematodes penetration and development was generally increased.

2. Materials and Methods

Nematode and fungi stock culture:

Meloidogyne incognita: Larvae of *M. incognit* were obtained by placing egg-masses upon nematode extraction tissue filter paper on a nylon- bottomed plastic ring touching the surface of water in plastic dish. These egg-masses were collected from the pure stock culture of *M.*

incognita maintained on tomato plants, Lycopersicon esculentum Mill, cv. Prichard.

***Rotylenchulus reniformis*:**
Egg-masses were collected from infected roots of soybeans. Immature females of the nematode were obtained by placing the egg-masses upon nematode extraction tissue filter paper on a nylon- bottomed plastic ring touching the surface of water in a plastic dish.

***Heterodera cajani*:**
Cysts *H. cajani* were collected from snap bean fields in Perkash, Giza governorate. A large number of nematode cysts were obtained by the previously described extracting method.

***Fusarium solani*:**
The isolated *F. solani* fungi were grown for 2 weeks at 30 ± 1 C° in bottles containing sterilized sand-barley medium (25 g clean sand, 75 g barley grains and enough water to cover the mixture) followed by sterilization at 15 Ib/in^2 for minutes. For soil infestation the culture contents of the two weeks old bottles were thoroughly mixed with sterilized soil at the rate of 5% the soil weight, and then filled in previously sterilized pots, the infested soils were moistened and mixed thoroughly every day for one week to ensure even growth and distribution of the inoculated fungi.
Micronutrients content (Zn, Mn, Fe, and Cu) were determined by using a pye unican Model sp, 1900 Atomic Absorption.

Pathogenesis of *H. cajani, M. incognita* and *Fusarium solani* on cowpea,
Two greenhouse experiments were conducted to study interactions among *H. cajani*, *R. reniformis* and Fusarium solani on cowpea plants.

Experiment 1: Concomitant inocula levels of *H. cajani M incognita*, and *R. reniformis* on cowpea.
Seeds of "Balady" cowpea were planted in 55 plastic 15 cm diameter pots filled with autoclaved sandy loam soil formed of 1 part sand+ 1 part loam and inoculated with the cowpea root modeling bacteria *Rhizobium leguminosarium.* After germination, cowpea seedlings were thinned to two plants per pot. Pots were, then divided into eleven groups representing treatments and each treatment comprised of 5 replicated pots as follows:-
Treatment (1): Nematode non-inoculated control.

Treatment (2): Inoculated with 1000jj *H. cajani* alone.

Treatment (3): Inoculated with 1000 jj *H. cajani*+ 1000 JJ *M. incognita*+ 1000 jj and young females *R. reniformis*.

Treatment (4): Inoculated with 2000 jj *H. cajani* alone;

Treatment (5): Inoculated with 2000 jj *H. cajani* + 1000jj *M. incognita*+ 1000jj and young females *R. reniformis*.

Treatment (6): Inoculated with 4000jj *H. cajani* alone.

Treatment (7): Inoculated with 4000 jj *H. cajani* + 1000 jj *M. incognita* + 1000 JJ and young females *R. reniformis*.

Treatment (8): Inoculated with 1000 jj *H. cajani*+ 2000jj *M. incognita*+ 1000 jj young females R. reniformis.

Treatment (9): Inoculated with 1000 jj H cajani+ 4000jj M incognita+ 1000jj young females *R. reniformis*.

Treatment (10): Inoculated with 1000 jj *H. cajani* + 1000jj *M. incognita* + 2000 jj and young females *R. reniformis*.

Treatment (11): Inoculated with 1000 jj *H. cajani*+ 1000 jj *M. incognita* + 4000 jj and young females *R . reniformis*.

All pots were arranged according to a randomized block design in a greenhouse on a daily temperature of 30±2°C. Cowpea plants were watered daily and treated according to the recommended agricultural practices for cowpea production and protection. The experiment lasted for 97 days, after which final population of each nematode species as well as adults, developmental stages cysts were counted in the soil and roots. Killing and fixation of plant roots were achieved by immersing in F.A.A. salaaming nematodes in plant tissue was made by incubating roots, for 3-4 min in lacto phenol Acid fuchsine solution (Franklin and Goodey, [12]). Nematode stages were counted using a dissecting microscope. At the end of the experiment, shoot height, root length, number of leaves, flowers, pods and seeds were recorded. Fresh and dry weight of shoots, roots, pods and seeds were determined. N, P and K percentages were determined in the roots, shoots and seeds according to the methods of Prygl [13], Troug and. Mayer [14] and Richards [15]respectively. Fe, Zn, Mn, and Cu contents in roots, shoots and seeds were determined on a Rue Unican Model sp 1900.

Experiment 2: Concomitant infections of *H. cajani*, *M. incognita*, *R. reniformis* and *F. solani*, on cowpea.

Seeds of cowpea cv. "Balady" were planted in 45 plastic 15-cms diameter pots of autoclaved sandy loam soil. After germination, pots were divided into nine treatments, each of five replications, as follows.

Treatment (1):*H. cajani* alone

Treatment (2):*H. cajani* + *M. incognita*

Treatment (3):*H. cajani* + *M. reniformis*

Treatment (4):*H. cajani* + *F. solani*

Treatment (5):*H. cajani* + *M. incognita* + *R. reniformis*

Treatment (6):*H.cajani* + *M.incognita* + *F.solani*

Treatment (7):*H.cajani* + *R. reniformis* + *F. solani*

Treatment (8):*H.cajani* + *M.incognita* + *R. reniformis* + *F. solani*

Treatment (9): Without nematodes and fungus as control.

Nematode inocula were 50 crushed *H. cajani* cysts per pot, 1250-second stage larvae *H. incognita* per pot, 1000 young females' larvae *R. reniformis* per pot and for *F. solani* was 32 g barley culture of the fungus. All pots were arranged according to a randomized block design in a greenhouse on a daily temperature of 32 ± 20°C. Pots were watered daily and treated according to the recommended agriculture practices for cowpea production and protection. The experiment lasted for 72 days, after which days, which final population of each nematode species as well as adults, developmental stages and cysts were counted in the soil and roots as described before. At the end of the experiment, shoot height, root length, number of leaves, and no. of flowers were recorded. Fresh and dry weight of shoots of shoots and roots were also determined. N, P, Fe, Zn, Mn and Cu were determined in the roots and shoots according to the methods used in experiment 1.

3. Results and Discussion

Effects of concomitant inocula of *H. cajani*, *M.incognita* and *R. reniformis* on cowpea.

Tables (1- 4) Shows the effects of different larval inocula of *H.cajani* alone, and combination with *M. incognita* and *R. reniformis* on nematode development (Table 1), on roots, shoots, leaves, flowers pods and seeds, and Table (2) on N, P, K, Fe, Zn, Mn, and Cu in roots, shoots and seeds of cowpea plants (Tables 3 and 4).

Table (1) shows that more eggs, L2 and cysts were developed in cowpea roots in the *H. cajani* alone treatments. The final cyst population- being, increased with the increase of inoculated larvae- is positively correlated with the larval inocula level of *H. cajani*. With the exception of the treatment 1000 Hc+ 4000 Mi+ 1000Rr, statistical more *H. cajani* L2 population were extracted from the roots of the concomitant nematode treatments than from *H. cajani* single inoculation treatment.

Table (2) shows that the non- infected cowpea plants have significantly more fresh and dry weight of roots or shoots than the 1000, 2000 or 4000 *H.cajani* larvae- infected cowpea with the exception of the 2000 larvae treatment which has no statistical difference in shoot dry weight than the non-infected plants. No statistical difference was also found between the healthy and the three *H. cajani* inocula levels in the final number of leaves or flowers. The table also shows that while pods and seeds were formed in the non- infected plants, no such organs were formed on any of the three inocula of *H. cajani* treatments. N, P, K, Zn, and Mn contents in roots (tables 3 and 4) were not affected in the single H. cajani treatments. Fe concentration in roots (Table 4) of the single 1000 jj *H. cajani* infection with the exception of 1000 Hc= 4000 Mi+ 1000 Rr. In all the concomitant inocula treatments no statistical differences among treatments occurred in fresh or dry weight of roots and shoots, number of leaves, flowers, number and dry weight of pods and number and dry weight of seeds (Table 2) and in N, P and K contents of roots, shoots or seeds (Table 3), the only Fe change was the statistical increase of shoot Fe in the 1000 Hc+ 1000 Mi+ 2000 Rr and in the 4000 Hc+ 1000Rr treatments. Shoot Zn was decreased statistically in all the concomitant inocula treatments than the 1000 Hc+ 1000 Mi+ 1000 Rr treatment. The treatment 1000 Hc+ 1000 Mi+ 2000Rr decreased Mn significantly in shoots or seeds, 411e the treatments 1000 Hc+ 1000 Mi+ 1000Rr decreased it in seeds (Table 4).

Effects of concomitant infections of *H. cajani* (Hc), *M. incognita* (Mi), *R. reniformis* (Rr) and *F. solani* (Fs) on cowpea and nematode development.

Table (5) shows that the final *H. cajani* cyst population was statistically fewer on cowpea roots infected with Hc+ Mi, Hc+ Mi+ Rr, Hc+ Mi+ Fs or Hc+ Mi+Rr+ Fs than Hc alone. This finding suggests that the infection of the two concomitant species *H. cajani* and *M.incognita* yield less *H. cajani* cysts than the infection with *H. cajani* alone . This result was found also true for the concomitant infections of *H.cajani* and *F.solani* treatments where Hc+ Fs, Hc+ Mi+ Fs, Hc+ Rr+ Fs or Hc+ Mi+ Rr+ Fs gave statistically less H cajani cysts than H cajani alone .

Table (5) also shows that while infection of Hc+ Rr did not give statistical difference with Hc alone, the treatments Hc+ Mi+ Rr, Hc+ Fs and Hc+ Mi+ Rr+ Fs gave statistically fewer cysts than Hc alone. When the different *M. incognita* treatments Hc+ Mi, Hc+ Mi+ Fs and Hc+ Mi+ Rr+ Fs were compared for the final number of *M. incognita* larvae in potted soil and for the number of females with and without eggs, Table (5) indicates that the only two statistical differences were between Hc+ Mi+ Rr+ Fs and Hc+ Mi in the number of larvae in potted soil and between Hc+ Mi+ Rr and the other three treatments in number of females with eggs. Not all the other comparisons were significant.

On the contrary, when the different *R. reniformis* treatments- Hc+ Rr, Hc+ Rr+ Fs and Hc+ Mi+ Rr+ Fs were compared for the final number of *R. reniformis* in soil and roots, Table 5 indicates that Hc+ Rr treatment gave statistically larger numbers or Rr soil and females with and without eggs than the other three treatments. Exceptionally, the number of Rr females with eggs in Hc+ Mi+ Rr+ Fs was statistically not different than the other three treatments.

Table 1. Effect of concomitant inocula levels of *H.cajani, M.incognita* and *R.reniformis* on the final populations of these nematodes in soils and roots of the infected cowpea plants.

Treatments			*H. cajani* (Hc)						Total roots Cyst	*M. incognita* (Mi)					*R. reniformis* (Rr)					Total roots
			Soil	0.5 g roots						Soil L2	0.5 g roots				Soil		0.5 g roots			
Hc	Mi	Rr	L2	Egg masses	L2	With	Cysts				L3	L4	Egg masses	Total roots	L2	Young			Egg masses	
0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
1000	0	0	0a	130a	104a	0a	9a	49a	0	0	0	0	0	0	0	0	0	0	0	0
1000	1000	1000	108a	0a	0b	La	2a	16b	661ab	0	0a	15an	13a	111ab	2846a	495a	690a	32a	27a	273
1000	1000	2000	88bc	0b	0b	2a	0a	0b	700ab	0	La	25a	11a	206a	5388ab	308a	1425a	105b	10a	906a
1000	1000	4000	117b	0b	0b	0a	La	10b	150a	0	0a	9b	6a	95ab	7500b	2863b	2675b	173c	38a	187
1000	2000	1000	50c	0b	0b	0a	0a	0b	114ab	0	3a	10b	2a	67b	4171ab	2692b	676a	81bd	4a	638a
1000	4000	1000	0a	0b	0b	0a	La	14b	1487b	0	2a	4b	2a	57b	3843ab	450a	639a	69d	15a	974a
2000	0	0	0a	30a	65a	0a	13a	35a	0	0	0	0	0	0	0	0	0	0	0	0
2000	1000	1000	226b	0a	0a	2a	16	7b	586	0	20	115	75	768	1521	489	624	173	54	115
4000	0	0	0a	43a	160a	0a	43a	100a	0	0	0	0	0	0	0	0	0	0	0	0
4000	1000	1000	149b	0a	0b	0	1b	0b	243	0	3	19	3	149	306	2565	2623	280	21	220

Figures with different latter are statistically different to according to Duncan's Multiple Range Test.

Table 2. Effects of concomitant inocula levels of *H.cajani, M.incognita* and *R.reniformis* on the lengths and weights of roots and shoots number of leaves and flowers and nos and dry weights of pods and seeds of the infected cowpea plants.

Treatment			Roots			Shoots			Leaves	Flowers	Pods		Seeds	
				Weights			Weights							
Hc	Mi	Rr	Length	Fresh	Dry	Height	Fresh	Dry			No	Dry wt.	No.	Dry wt
0	0	0	22.50a	7.39a	2.34a	36.63ab	18.62a	3.67a	4a	2a	1.00a	0.29a	3.00a	0.24a
1000	0	0	20.33a	2.70b	6.40b	16.67d	4.80b	0.90b	4a	1a	0.00a	0.00a	0.00a	0.00a
1000	1000	1000	11.75a	3.75ab	0.77b	43.88ab	12.36ab	3.24a	7a	1a	0.37a	0.16a	1.00a	0.17a
1000	1000	2000	11.17a	4.02ab	0.67b	50.06bc	8.54ab	2.68a	6a	2a	0.17a	0.09a	0.25a	0.08a
1000	1000	4000	6.79a	4.85ab	1.75ab	52.50c	11.69ab	3.00a	5a	1a	0.14a	0.13a	1.00a	0.10a
1000	2000	1000	9.75a	3.93ab	1.05ab	32.05a	8.45ab	2007a	5a	0a	0.33a	0.08a	1.00a	0.07a
1000	4000	1000	11.88a	6.90ab	2.31ab	40.69abc	14.79ab	3.79ab	7a	2a	0.13a	0.04a	1.00a	0.03a
2000	0	0	16.33a	1.35b	0.36b	11.00b	3.48b	1.09a	2a	1a	0.00	0.00	0.00	0.00
2000	1000	1000	6.60a	3.34ab	0.59b	40.63a	6.79ab	2.51a	4a	0a	0.00	0.00	0.00	0.00
4000	0	0	23.42a	1.16b	0.29b	19.33b	3.55b	0.69b	3a	0a	0.00	0.00a	0.00	0.00
4000	1000	1000	9.00a	3.39ab	0.58b	33.31ab	13.35ab	2.62ab	5a	1a	1.00	0.00a	1.00a	0.00

Figures with different letters are statistically different according to Duncan's Multiple Range Test.

Table 3. Effects of concomitant inocula levels of *H.cajani, M.incognita* and *R.reniformis* on N, P and K in roots, shoots seeds of the infected cowpea plants.

Treatments			N%			P%			K%		
Hc	**Mi**	**Rr**	**Roots**	**Shoots**	**Seeds**	**Roots**	**Shoots**	**Seeds**	**Roots**	**Shoots**	**Seeds**
0	0	0	8.32a	12.01a	18.42a	0.14a	0.12a	0.22a	0.81a	1.04a	0.65a
1000	0	0	6.06a	0.00	0.00a	0.30a	0.00	0.00	0.36a	0.00	0.00
1000	1000	1000	11.88a	6.35a	1.62a	0.30a	0.29a	0.42a	0.59a	1.23a	0.92a
1000	1000	2000	5.59a	7.28a	0.81a	0.25a	8.15a	0.42a	0.21a	1.17a	0.72a
1000	1000	4000	9.15a	12.15a	1.51a	0.25a	0.19a	0.59a	0.69a	1.04a	0.72a
1000	2000	1000	9.29a	8.80a	1.40a	0.30a	0.35a	0.54a	0.34a	1.04a	0.72a
1000	4000	1000	8.94a	7.44a	1.42a	0.21a	0.31a	0.74a	0.44a	1.08a	0.67a
2000	0	0	11.82a	0.00	0.00	0.34a	0.00	0.00	0.31a	0.00	0.00
2000	1000	1000	6.79a	5.33	0.00	0.27a	0.14	0.00	0.28a	1.17	0.00
4000	0	0	9.17a	0.00	0.00	0.98a	0.00	0.00	0.19a	0.00	0.00
4000	1000	1000	9.70a	11.19	1.86	0.26a	0.39	0.76	0.55a	1.13a	0.73

Figures with different letters are statistically different according to Duncan's Multiple Range Test

Table 4. Effects of concomitant inocula levels of *H.cajani, M.incognita* and *R.reniformis* on Zn, Fe and Cu in roots, shoots seeds of the infected cowpea plant.

Treatments			Zn (mg/kg Dw.)			Mn (mg/kg Dw.)			Fe (mg/kg Dw.)			Cu (mg/kg Dw.)		
Hc	**Mi**	**Rr**	**Roots**	**Shoots**	**Seeds**	**Roots**	**Shoots**	**Seeds**	**Roots**	**Shoots**	**Seeds**	**Roots**	**Shoots**	**Seeds**
0	0	0	94.17a	67.50ab	70.01a	69.17a	52.08a	15.00a	5616.78ac	362.50a	117.20a	81.67a	35.56a	41.67a
1000	0	0	90.23a	0.00	0.00a	74.16a	0.00	0.00	3726.25a	0.00	0.00	0.00	0.00	0.00
1000	1000	1000	208.34a	77.11b	58.34a	139.17a	30.00a	8.33b	6413.41cb	505.85a	63.34a	33.33a	25.00a	81.67a
1000	1000	2000	87.71a	41.11a	81.67a	115.29a	21.14b	1.67c	9176.26b	1019.19b	0.00	49.59a	48.89a	48.33a
1000	1000	4000	68.34a	37.92a	61.67a	78.35a	36.25ab	5.00b	6899.31cb	244.45a	0.00	16.69a	41.11a	8.33a
1000	2000	1000	138.79a	40.42a	71.67a	130.71a	45.00	5.00b	7065.76cb	791.12a	111.67a	0.00a	65.00a	0.00
1000	4000	1000	82.72a	39.17a	64.17a	80.92a	44.58ab	1.67c	5081.72ac	597.51a		48.42a	32.76a	33.33a
2000	0	0	124.55a	0.00	0.00	88.70a	0.00	0.00	5212.23a	0.00	0.00	0.00	0.00	0.00
2000	1000	1000	136.96a	55.42	0.00	83.59a	29.59	0.00	6129.76a	294.58	0.00	54.59a	37.67	0.00
4000	0	0	109.73a	0.00	0.00	106.06a	0.00	0.00	7226.59a	0.00	0.00	0.00	0.00	0.00
4000	1000	1000	88.10a	43.33	87.51	43.03a	74.59a	8.33	6433.72a	987.93	0.00	90.00a	25.00a	53.34

Figures with different letters are statistically different according to Duncan's Multiple Range Test.

Table 5. Effects of concomitant infection of *H.cajani, M.incognita* and *R.reniformis* and *F.solani* on the final populations of these nematodes in soil and roots of the infected cowpea plants.

Treatment	*H.cajani* (Hc)				M. incognita (Mi)				R. reniformis (Rr)			
	Soil		0.5g roots	Total Roots	Soil	0.5g Roots	Egg-masses	Total roots	Soil	0.5g roots	Egg-masses	Total roots
	Cyst	Pf/Pi	Cyst	Cyst	L2							
Hc	391a	7.80	8b	73b	0	0	0	0	0	0	0	0
Hc +Mi	188bc	3.70	4ab	45ab	126a	18a	8a	202a	0	0	0	0
Hc +Rr	261ab	5.22	2a	23a	0	0	0	0	9265a	520a	80a	7130a
Hc + Fs	158bc	3.15	6ab	87b	0	0	0	0	0	0	0	0
Hc +Mi + Rr	88bc	1.75	3ab	27a	244ab	24a	9a	218a	375b	43b	18b	2093b
Hc + Mi + Fs	137bc	2.73	1a	18a	142ab	8a	5a	144a	0	0	0	0
Hc + Rr+ Fs	42c	0.84	2a	19a	0	0	0	0	146b	26b	16b	253c
Hc+Mi + Rr + Fs	91bc	1.82	1a	8a	305b	15a	5a	119a	363b	67b	32ab	533c

Figures with different letters are statistically different according to Duncan's Multiple Range Test.

Table 6. Effects of concomitant infection of *H.cajani, M.incognita* and *R.reniformis* and *F.solani* on the infected cowpea plants.

Treatments	Roots			Shoots		
	Length	Weight		Height	Weight	
		Fresh	Dry		Fresh	Dry
Hc	26.60ab	4.57a	1.82a	31.31a	4.80ad	2.36a
Hc +Mi	27.20ab	5.62a	1.95ad	33.76ab	13.85b	3.23a
Hc +Rr	24.78ab	5.75a	1.55c	36.57ab	9.69abd	3.12a
Hc + Fs	22.80ab	7.24a	2.44b	51.10a	22.01c	7.17b
Hc +Mi + Rr	29.80b	4.55a	1.43c	38.56ab	12.35bd	3.47a
Hc + Mi + Fs	29.56b	8.98b	2.17bd	41.50ab	21.81c	5.33c
Hc + Rr+ Fs	24.13ab	4.86a	2.06ad	45.31ab	28.28c	6.49c
Hc+ Mi + Rr + Fs	16.43ab	3.98a	2.13ab	42.70ab	27.88c	5.62c
Control	27.50ab	2.14a	1.30c	30.33b	6.22d	2.53a

Figures with different letters are statistically different according to Duncan's Multiple Range Test.

By comparing the nematode- infected and non- infected control, Tables (6), and (7) and (8) show that:

1. Hc- infected plants had statistically larger root dry weight shoot height and root K:
2. Hc+ Mi treatments had statistically more root dry weight, shoots fresh weight, and more root K:
3. Hc+ Rr treatments had statistically more leaves, less roots N, more shoot N and P and more roots Fe and Zn:
4. Hc+ Fs- infected plants had statistically more root dry weight, higher shoots, more shoot fresh weight, more leaves, more flowers and more root N and K:
5. Hc+ Mi+ Rr treatments had statistically less root N, more shoot P, more root and shoot K and more shoot Zn:
6. Hc+ Mi+ Fs treatments had statistically more root, fresh weight more root dry weight, more shoot fresh weight, more shoot dry weight more leaves, less root N and more root and Zn:
7. Hc+ Rr+ Fs treatments had statistically more root dry weight, more shoot fresh weight, more shoot dry weight, more leaves, less root N and more shoot Zn:
8. Hc+ Mi Rr+ Fs plants had statistically more root, fresh weight more shoot fresh weight, more shoot dry weight, more leaves, less root N and more root Zn.

Similarly, comparisons among the different treatments in Tables (6), (7) show the following findings:

1. Hc+ Mi had statistically higher shoot and root phosphorus than He alone.
2. Hc+ Rr had a statistically more number of leaves, shoot nitrogen, shoot phosphorus, root and shoot iron and root zinc, but lower root dry weight and root N and P than He only .
3. Hc= Fs had statistically more dry weight, shoot fresh weight, shoot dry weight, number of leaves and no, of flowers, but less root or shoot N, and root potassium than He only.
4. Hc+ Rr differs statistically than Hc+ Mi in having less root dry weight, more leaves, more shoot P, less root K, more root and shoot Fe and more root Zn.
5. Hc+ Fs differs statistically than Hc+ Mi in having more root dry weight, more shoot fresh weight, more shoot dry weight, more leaves, less root and N and root K.
6. Hc+ Mi+ Rr had statistically less root dry weight and more shoot phosphorus and zinc Hc+ Mi.
7. Hc+ Mi+ Rr had statistically more root fresh weight, shoot fresh weight, shoot dry weight, number of leaves, root, and shoot zinc less root potassium, than Hc+ Mi.
8. Hc+ Mi+ Rr differs statistically than Hc+ Rr in having less shoot N, more root and shoot K and less root Fe.
9. Hc+ Rr+ Fs differs statistically than Hc+ Rr in having more R. Dr. Wt, more Sh. Fr. Wt, more Sh. Dr. Wt, more leaves, less shoot N, less, shoot P, less root and shoot Fe and less root and shoot Zn.
10. Hc+ Mi+ Rr+ Fs differs statistically than Hc+ Rr in having more root dry weight, more shoot fresh weight, more shoot dry weight, more leaves, less shoot N, less shoot P and less root Fe.
11. Hc+ Mi+ Fs differs statistically than Hc+ fs in having less Shoot dry weight, more root N, more root K, and more root Zn.
12. Hc+ Rr+ Fs differs statistically than Hc+ Fs in having less root dry weight, less shoot dry weight, fewer leaves, more root N, more K, less root Fe and more shoot Zn.
13. Hc+ Mi+ Fs differs statistically than Hc+ Mi + Rr in having more root fresh weight, root dry weight, shoot fresh weight, shoot dry weight, number of

leaves, more root and shoot zinc and fewer shoot P and root and shoot K.

14. Hc+ Rr+ Fs differs statistically than Hc + Mi + Rr in having more root dry weight, shoot fresh weight, more shoot dry weight, more leaves, less shoot, P, less root K. and less shoot K.
15. Hc+ Rr+ Fs differs statistically than Hc+ Mi+ Fs in having less root fresh weight, and less root Zn.
16. Hc+ Mi+ Rr+ Fs differs statistically than Het Mi+ Rr in having statistically more root, fresh weight, but less root length shoot, fresh weight, shoot dry weight, number of leaves, shoot phosphorus and root and shoot potassium.
17. Hc+ Mi+ Rr+ Fs differs statistically than Hc+ Mi+ Fs in having statistically less root length, root fresh weight and root zinc.
18. Hc+ Mi+ Rr+ Fs differs statistically than Hc+ Rr+ Fs in having more roots Zn and less shoot.

Table 7. Effects of concomitant inocula level of *H.cajani, M.incognita* and *R.reniformis* and *F.solani* on Zn, Mn, Fe and Cu in roots, shoots and seeds of the infected cowpea plant.

Treatment	N%		P%		K%	
	Roots	Shoots	Roots	Shoots	Roots	Shoots
Hc	1.66a	2.78a	0.30a	0.18a	1.40a	1.65a
Hc +Mi	1.46ab	3.09ab	0.72ab	1.02a	1.54a	2.30ab
Hc +Rr	1.18bd	3.66b	0.60ab	2.62b	1.25c	1.50a
Hc + Fs	0.72c	1.92c	0.35ab	0.53a	0.70b	1.64a
Hc +Mi + Rr	1.30bd	2.79a	0.53ab	2.01b	1.57a	2.67b
Hc + Mi + Fs	1.27bd	2.45ac	0.61ab	0.77a	1.26c	1.42a
Hc + Rr+ Fs	1.12d	2.44ac	0.48ab	0.79a	1.28c	1.59a
Hc+ Mi + Rr + Fs	1.13d	2.43ac	0.58ab	0.87a	1.33c	1.50a
Control	1.63a	2.51ac	0.40ab	0.23a	1.22c	1.72a

Figures with different letters are statistically different according to Duncan's Multiple Range Test.

Table 8. Effects of concomitant inocula level of *H.cajani, M.incognita* and *R.reniformis* and *F.solani* on Zn, Mn, Fe and Cu in roots, shoots and seeds of the infected cowpea plant.

Treatment	Zn (mg/kg Dw.)		Mn (mg/kg Dw.)		Fe (mg/kg Dw.)		Cu (mg/kg Dw.)	
	Roots	Shoots	Roots	Shoots	Roots	Shoots	Roots	Shoots
Hc	59.2a	41.7a	201a	167a	1159a	75a	34.2a	25a
Hc +Mi	91.7abc	46.7a	343a	230a	1859ac	100a	45.0a	33.4a
Hc +Rr	137.5bc	62.5ab	246a	483a	2492b	533b	53.4a	35.0a
Hc + Fs	100.0ab	59.1ab	487a	183a	2508ab	92a	45.0a	26.7a
Hc +Mi + Rr	100.0ab	83.3bc	300a	257a	1725ac	200ab	45.8a	26.7a
Hc + Mi + Fs	200.0c	79.2bc	255a	182a	1925ac	358ab	51.7a	34.2a
Hc + Rr+ Fs	63.4a	95.8c	172a	170a	59c	33a	35.9a	35.0a
Hc+ Mi + Rr + Fs	104.0b	58.3ab	151a	213a	106c	142ab	44.2a	33.0a
Control	45.0a	29.6a	249a	127a	1434ac	400ab	41.7a	26.7a

Figures with different letters are statistically different according to Duncan's Multiple Range Test.

Interaction cases among cyst, root knot, reniform nematodes and fungi attaching common hosts have been reported in this study and by many authors (Polychronospoulos et al. [7] Meagher and Chambers [8] Sharma and Sethi [16,1,2] Jatala and Jensen [4,5] Meagher et al, [9] Gill and Swarup [10] Massoud [3] Nordmeyer and Sikora [11] and Niblack *et al.* [6]). In these reports and in the present study, significant mutual influences concerning the development and final populations of these concomitant nematodes have been confirmed. The final population of *H. cajani* increased when its initial population increased at inocula of 2000 jj2 at inoculum of jj2 *H. cajani*, final population of *M. incognita* decreased, while the final population of *R. reniformis* increased. When the initial population of *M. incognita* were at inocula of 2000 and 4000 jj2, the final population of *M. incognita* and *R. reniformis* increased while the population of *H. cajani* markedly decreased. When the initial population of *R. reniformis* was at inoculums of 4000 (young female+ jj2). Its final population increased drastically.

In general, when *H. cajani*, *M. incognita* and *R. reniformis* were simultaneously inoculated, they decreased all studied plant growth criteria except the height of shoots.

Our work indicated that the lowest numbers of cysts were obtained from *H. cajani+ R. reniformis + F. solani* in soil and roots. Multiplication of cyst nematodes was lower in the presence of *F. solani* alone. These results confirm the previous findings by Kumar *et al.* [17].Who found low multiplication of root knot nematode in the presence of the fungus *F. oxysporum*. Mani and Sethi, [18]also found low multiplication of root knot due to the possible existence of nematoxic anti-metabolites produced by the fungus, *F.oxysporum*.

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