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STUDIES ON ROOT-KNOT, ROOT-ROT AND PEA MOSAIC VIRUS COMPLEX OF *PISUM SATIVUM*

by
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A survey of pea (*Pisum sativum* L.) crops growing in the vicinity of Aligarh, U. P., India, revealed that they were frequently infected with pea mosaic virus, the roots heavily galled by *Meloidogyne incognita* (Kofoid *et* White) Chitw. and infected with *Rhizoctonia solani* Kuen. The plants were dwarfed and very poor root nodule development was observed. It was, therefore, considered interesting to study the inter-relationship of pea mosaic virus, *M. incognita, R. solani* and *Rhizobium* sp. causing a disease complex in pea.

Materials and Methods

Surface sterilized and bacterized (with *Rhizobium* pea strain) pea seeds cv. Sutton Phenomenon were sown in 15 cm clay pots containing autoclaved soil mixture of sand, soil and compost (1:2:1). The virus isolate from naturally infected plants and maintained in pea, was sap inoculated to seedlings. Nematode inoculations were made with 2000 freshly hatched larvae of *M. incognita* and fungal inoculations with 1g fungus per plant. One week old seedlings were used for all treatments. Each treatment was replicated five times. Experiments were carried out in an insect proof glass house at 15-20 °C. Individual and concomitant inoculations with the test pathogens were made as shown in Table 1.

The plants were uprooted after sixty days and their roots gently washed. The length of plants was measured and roots and shoots were weighed. Nematodes were extracted from the soil using Oostenbrink's elutriator and Baerman funnels and from roots by comminuting in a Waring blender. Root-knot galling and root-nodulation indices were estimated on the basis of the following.

Root-knot Index Scale		Root-nodule Index Scale	
Galling	Index	Nodulation	Index
No galling	0	Nil	0
1-25 galls/plant	1	Few	1
26-50 galls/plant	2	Light	2
51-75 galls/plant	3	Moderate	3
76-100 galls/plant	4	Heavy	4
101 and above galls/plant	5		

Multiplication rate of nematodes was determined by calculating the reproductive factor ($R = \frac{Pf}{Pi}$), where Pf means final and Pi initial populations of nematodes respectively.

Results

Data presented in Table I clearly show that all three test organisms (*M. incognita, R. solani* and Pea mosaic virus), both singly or in their various combinations, caused significant damage to *P. sativum* cv. Sutton Phenomenon. Individually, however, virus infection caused greater reduction in the shoot length as well as in dry weight of root and shoot than the fungus or nematode. The combination of two or three of the pathogens caused much more damage than caused by either pathogen alone.

Simultaneous inoculations of pea plants with the nematode, fungus and the virus caused the greatest damage, reducing root-shoot length by 58% and root-shoot dry weight by 64% (Table I). Nematode-virus combination, irrespective of inoculation timings, caused more plant growth reduction than nematode-fungal inoculation. However, the greatest reduction in plant length and dry weight was caused by simultaneous inoculation of virus and nematodes. Inoculation with nematodes followed by virus inoculation 10 days later caused more damage than inoculation with the virus followed by nematodes 10 days later. Similarly, percentage growth reduction was greater in plants simultaneously inoculated with nematode and fungus, than when fungus or nematodes were inoculated at intervals of 10 days (Table I).

With virus-fungus combinations the greatest reduction in plant growth occurred when fungus inoculation preceded virus inoculation, while it was least when virus inoculation preceded fungus inoculation.

Nematode multiplication was least (R = 3.8) on plants simultaneously inoculated with all three test pathogens. All combinations of nematode-fungus inoculations produced an inhibitory effect on nematode multiplication, while simultaneous inoculations with nematode and virus or nematode inoculation followed by virus 10 days later, considerably increased nematode multiplication. Inoculation with virus followed by nematodes 10 days later, on the other hand, inhibited nematode multiplication.

Poorest root-knot development (RKI = 1.2) was observed on plants simultaneously inoculated with all three pathogens. Root-knot development was poor when virus or fungus inoculations preceded nematode inoculation, as also in the case of simultaneous inoculation with fungus and nematode. Prior inoculation with fungus was, however, more inhibitory to root-knot development than prior inoculation with virus.

Nodulation was also suppressed by the simultaneous inoculation with all three pathogens. Virus alone had little adverse effect on nodulation but simultaneous inoculation with nematode and fungus as well as inoculation with fungus alone or in different combinations with virus or nematode suppressed nodulation. The fungus (*R. solani*) thus was inhibitory both to nodulation and root-knot development.

Discussion

Percentage growth reduction was highest when plants were simultaneously inoculated with all three organisms because of their synergistic effect. Severely damaged root systems due to combined infection of *M. incognita* and *R. solani* were possibly responsible for

Table I - *Individual and concomitant effect of* Meloidogyne incognita, Rhizoctonia solani *and pea mosaic virus on nematode multiplication, root-knot development, nodulation and plant growth of* Pisum sativum *cv. Sutton Phenomenon.*

Treatment	Length in cm			Percent reduction in length against control	Dry weight in g			Percent reduction in weight against control	Larval population in soil/kg	Nematode population (all stages per root-system)	Total nematode population in soil and root	$R = \frac{Pf}{Pi}$	Root knot index	Nodule index
	Shoot	Root	Total		Shoot	Root	Total							
CONTROL (No pathogen)	36	14	50	—	8.3	3.8	12.1	—	—	—	—	—	—	3.8
Virus alone	26	11	37	26	5.8	2.7	8.5	29.7	—	—	—	—	—	3.4
Nematode alone	33	10	43	14	7.6	3.2	10.8	10.7	8293	185	8478	8.5	3.8	3.0
Fungus alone	29	10	39	22	6.5	2.6	9.1	24.8	—	—	—	—	—	2.6
Nematode and virus simultaneously	16	8	24	52	3.3	2.0	5.3	56.2	11824	215	12039	12.0	4.2	2.8
Virus inoculation 10 days after nematode inoculation	20	11	31	38	5.2	2.1	7.3	39.7	9676	194	9870	9.9	4.0	2.9
Nematode inoculation 10 days after virus inoculation	20	9	29	42	4.1	2.1	6.2	48.8	5934	108	6042	6.0	2.7	3.2
Nematode and fungus simultaneously	22	7	29	42	4.3	2.2	6.5	46.3	6853	123	6976	6.9	2.4	1.6
Nematode inoculation 10 days after fungus inoculation	21	10	31	38	4.8	2.3	7.1	41.3	4896	73	4969	4.9	1.5	1.9
Fungus inoculation 10 days after nematode inoculation	24	8	32	36	5.4	2.0	7.4	38.8	7218	132	7341	7.3	3.0	2.3
Virus and fungus simultaneously	24	11	35	30	5.7	2.3	8.0	33.9	—	—	—	—	—	3.1
Fungus inoculation 10 days after virus inoculation	25	11	36	28	5.8	2.4	8.2	32.2	—	—	—	—	—	3.2
Virus inoculation 10 days after fungus inoculation	23	8	31	38	4.5	2.1	6.6	45.4	—	—	—	—	—	2.8
Virus, fungus and nematode simultaneously	14	7	21	58	2.8	1.5	4.3	64.5	3764	54	3818	3.8	1.2	1.0
C.D. at 5% level	2.416	1.873	—	—	0.503	0.443	—	—	—	—	841	—	0.281	0.302
C.D. at 1% level	3.228	2.501	—	—	0.672	0.591	—	—	—	—	1124	—	0.376	0.403

Each value is an average of five replicates.

highly reduced nematode multiplication, poor galling and nodulation. Similarly, fungus inoculation preceding nematodes reduced nematode multiplication and galling because the nematodes failed to obtain sufficient nutrients from root tissues already damaged by prior fungal infection. This is supported by the fact that in the case of simultaneous or nematode inoculation preceding fungus, the nematode multiplication and root-knot index were not particularly low because the nematode inoculation preceding fungus allowed sufficient time for nematodes to establish themselves before the roots became damaged by the fungus. Goswami *et al.* (1975) also observed wilting in tomato plants when inoculated with *M. javanica* 3 weeks prior to inoculation with *R. bataticola*, while in simultaneous fungus plus nematode, fungus alone, or fungus 3 weeks prior to nematode inoculation, wilting was much less, thus indicating the antagonistic effect of fungus on nematodes. Chhabra *et al.* (1977) and Golden and Van Gundy (1975) obtained similar results with *M. incognita* and *R. solani* on different crops.

Nematode-virus combinations were more damaging than nematode-fungus or virus-fungus combinations because of the different sites of primary infection of the two pathogens and the antagonistic effect of virus on fungus. There was increased nematode multiplication and severe root galling when plants were either simultaneously inoculated with the nematode and virus or nematode inoculation preceded virus. This would be expected as the nematodes would have access to an undamaged site of infection. Swarup and Goswami (1969) also recorded significant increases in nematode populations in simultaneous infections with root-knot nematode and leaf curl virus in tomato. Goswami and Chenulu (1974) and Khurana *et al.* (1970) recorded similar results with other viruses and nematodes. On the other hand, virus inoculation preceding nematodes probably increased the rate of plant respiration and reduced the photosynthetic activity thus hampering proper transport and accumulation of food material in the nematode created metabolic sinks (giant cells) which are essential for nematode development and multiplication. This explains the reduced nematode multiplication and low root-knot index in this treatment.

Concomitant inoculation of pea mosaic virus and *R. solani* had little effect on plant growth, but pre-inoculation with the virus inhibited fungal development. This substantiates the findings of Chadha and Raychaudhuri (1966 and 1968) and Mahmood *et al.* (1974), who reported that infection of a plant with one pathogen affords partial or total protection against secondary infection with another pathogen. Chadha and Raychaudhuri (1966) reported inhibition of spore germination and subsequent retarded development of *Fusarium udum* on PDA medium containing extracts of pigeon pea infected by sterility virus. On the other hand, the increased reduction in plant growth when fungus inoculation preceded virus is attributable to the rotting of the root system having started before the virus inoculation.

SUMMARY

Pea mosaic virus caused more damage to *Pisum sativum* cv. Sutton Phenomenon than either *Meloidogyne incognita* or *Rhizoctonia solani.* All three pathogens individually suppressed nodulation but *R. solani* had the greatest effect. Greatest plant growth reduction but least nematode mutiplication, root-knot development and nodulation were caused by simultaneous inoculations with all three pathogens. Nematode-virus combinations were more damaging than nematode-fungus combinations. Simultaneous inoculation with either nematode-virus or nematode-fungus and nematode inoculation preceding either virus or fungus caused more damage than prior inoculations either with fungus or virus followed by nematodes 10 days later. *M. incognita* and *R. solani,* together, acted synergistically to reduce plant growth. *R. solani,* singly or in different combinations with nematodes, and prior virus inoculation followed by nematodes 10 days later, produced an inhibitory effect on nematode mutiplication, root-knot development and nodulation while simultaneous nematode-virus inoculation or nematode inoculation followed by virus 10 days later significantly increased nematode multiplication and root galling. Virus-fungus combinations caused greater damage when fungus inoculation preceded virus but was reversed when virus preceded fungus inoculation. Virus-fungus combinations showed a slight additive effect on plant growth reduction but the virus inhibited fungus development and thus its pathogenic effect.

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