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Interaction of *Meloidogyne incognita* and *Ralstonia solamacearum* on brinjal (*Solanum melongena* L.)

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ABSTRACT

The combined pathogenic effects of *Meloidogyne incognita* and *Ralstonia solanacearum* on brinjal were greater than independent effects of either. *M. incognita*, 15 days prior to the inoculation of *R. solanacearum* led to maximum wilt incidence (100%) followed by combined inoculation of both the pathogens simultaneously (75%). Low wilt incidence was noticed in plants inoculated with bacterium alone (50%).

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INTRODUCTION

Brinjal (*Solanum melongena* L.) also known as egg plant belongs to the family Solanaceae. Like other economically important crops, brinjal is also susceptible to many diseases caused by various agents *viz.,* fungi, viruses, bacteria, nematodes and phanerogamic parasite (*Orobanche* sp.). Root-knot nematode (*Meloidogyne incognita*) is one of the most potential plant parasitic nematodes which causes about 27.30-32.00 per cent yield loss on brinjal (Hazarika, 2003). Bacterial wilt of brinjal caused by *Ralstonia solanacearum* is the most prominent disease and could cause up to 80 to 90 per cent loss in yield (Kishun, 1980 and Rao *et al.,* 1975). It has been known for long time that root knot nematode facilitates entry and establishment of pathogenic fungi and bacteria (Powell, 1971). In the present study, efforts have been made to determine their combined effect on disease development and extent of damage caused by them in brinjal.

MATERIAL AND METHODS

Ralstonia solanacearum was isolated on Tetrazolium chloride (TZC) medium from wilted brinjal plants (Kelman, 1954). The bacterial suspension of virulent isolate was grown on Nutrient agar (NA) slants, incubated at 30°C. The pathogenic level for *R. solanacearum* was prepared by pouring sterile distilled water over 24 hrs old bacterial growth on NA slants and incubated for 1 hr to allow the viable bacterial cells to diffuse into the water then adjusting the optical density (OD) of bacterial suspension at 0.5 in spectrophotometer in blue filter (425 nm) to obtain a bacterial population of 1×10^8 colony forming units per milliliter (cfu/ml). *M. incognita* was maintained on tomato cultivar Pusa Ruby. Uniform sized egg masses from infested roots were picked and surface sterilized with 0.02 per cent ethyle mercuric chloride. The egg masses were rinsed with sterile water, kept in water in glass vials and inoculated through the plastic inoculation tubes. 15 cm diameter earthen pots were filled with 1 kg sterilized soil. Twenty-three days old seedlings of brinjal cv. Pusa Purple Long (PPL) were transplanted in each plot and regular watering was done to establish the seedlings well.

Ten ml of the nematode suspension containing about

1000 active *M. incognita* J2 was inoculated to 23 days old brinjal seedlings planted in 15 cm pots. The plastic tubes (10× 2 cm) were placed in pots adjacent to the seedling with the ends of tubes in contact with roots. 60 ml of bacterial suspension was dispensed per pot through the inoculation tubes there by reducing the possibility of wounds other than resulting from normal plant growth. The different treatments were as follows :

The treatments included (N) - Nematode alone, (B)- Bacterium alone, (NB)- Simultaneous inoculation of *M. incognita* and *R. solanacearum*, (Nb) -Nematode inoculation two weeks prior to bacterial inoculation, (Bn) - Bacterial inoculation two weeks prior to nematode inoculation and (C) - Un-inoculated control.

The experiment was monitored regularly and wilt symptoms were recorded at 25, 35 and 45 days interval. Plant showing wilt symptoms were given the score of 100 per cent PWI and 0 per cent PWI for plants as not showing any wilt symptom. After 45 days of inoculation, the experiment was terminated and observations were recorded. The individual plant was uprooted from pots carefully and washed gently in running tap water to remove the adhering soil particles from the roots. Care was taken so as to get the root system intact to avoid root damage. The observations were recorded on shoot length, fresh and dry weight of shoots and roots, number of galls and number of egg masses.

RESULTS AND DISCUSSION

Wilt symptoms were noticed 25 days after bacterial inoculation in the treatments where nematode was introduced 15 days prior to bacterial inoculation, whereas, in case of simultaneous inoculations, the plants wilted at 35 days. In other treatments, symptoms were observed after 45 days when maximum percentage (100%) was recorded in nematode inoculation followed by bacterial inoculation after 15 days intervals (Table 1). There was no difference in wilt incidence in treatments where bacterium alone (B) or bacterium inoculated prior to nematode inoculation (Bn). Similar results of increased bacterial wilt severity in the presence of *Meloidogyne* spp. was reported earlier (Sitaramaiah and Sinha, 1984; Libman *et al.,* 1964; Jatala *et al.,* 1975). Minimum wilt was noticed with bacterium alone which was on par with bacterium inoculated before the nematode. It has been known for a long time that root-knot nematode facilitates entry and establishment of pathogenic fungi and bacteria (Powell, 1971). In brinjal, the disease complex has been observed frequently involving *R. solanacearum* along with root-knot nematode contributing to an increase in wilt root-knot nematode contributing to an increase in wilt development (Swain *et al.,* 1987). The combined pathogenic effects of *Pseudomonas solanacearum* biotype–3 and *Meloidogyne javanica* on brinjal were greater than independent effects of either (Ravichandra *et al.,* 1990).

Table 1 : Effect of *M. incognita* and *R. solanacearum* individually and in combination on wilt incidence in brinjal cv. Pusa purple long

Treatments	Wilt incidence (%) Intervals of observation		
	25 DAI	35 DAI	45 DAI
N: Nematode alone (*Meloidogyne incognita*)	0.00	0.00	0.00
B: Bacterium alone *(Ralstonia solanacearum*)	0.00	0.00	50.00
Nb: Nematode inoculation followed by bacterium after 15 days	25.00	75.00	100.00
Bn: Bacterium inoculation followed nematode after 15 days	0.00	0.00	50.00
NB: Simultaneous inoculation of nematode and bacterium	0.00	25.00	75.00
C:Uninoculation control	0.00	0.00	0.00

Table 2 : Effect of *M. incognita* and *R. solanacearum* individually and in combination on development of *M. incognita* in brinjal cv. Pusa purple long

Treatments	No. of galls per plant	No. of egg masses per root	Root-knot index (0-5 Scale)
N: Nematode alone (*Meloidogyne incognita*)	178.75 (13.36)	132.25 (11.5)	5
B: Bacterium alone *(Ralstonia solanacearum*)	0.00(0.00)	0.00 (0.00)	0
Nb: Nematode inoculation followed by bacterium after 15 days	104.25 (10.21)	83.50 (9.13)	5
Bn: Bacterium inoculation followed nematode after 15 days	62.50 (7.90)	50.50 (7.10)	4
NB: Simultaneous inoculation of nematode and bacterium	75.75 (8.70)	66.25 (8.13)	4
C: Uninoculation control	0.00 (0.00)	0.00 (0.00)	0
S.E. ±	5.07	1.68	
C.D. (P=0.05)	15.06	5.00	
C.V. (%)	14.44	6.07	

Table 3 : Effect of *M. incognita* and *R. solanacearum* individually and in combination on growth parameters of brinjal cv. Pusa purple long

Treatments	Plant height (cm)*	Shoot weight (g)*		Root weight (g)*	
		Fresh	Dry	Fresh	Dry
N: Nematode alone (*Meloidogyne incognita*)	12.68 (3.56)#	7.90 (2.81)	3.06 (1.74)	3.90 (1.97)	1.53 (1.23)
B: Bacterium alone *(Ralstonia solanacearum*)	15.8 (3.97)	10.71 (3.27)	4.20 (2.04)	4.62 (2.14)	1.85 (1.36)
Nb: Nematode inoculation followed by bacterium after 15 days	8.40 (2.89)	6.22 (2.49)	2.44 (1.56)	2.58 (2.14)	1.06 (1.02)
Bn: Bacterium inoculation followed nematode after 15 days	10.40 (3.22)	7.97 (2.82)	3.22 1.79)	3.89 (1.97)	1.49 (1.22)
NB: Simultaneous inoculation of nematode and bacterium	10.80 (3.28)	8.02 (2.83)	2.82 (1.67)	3.19 (1.78)	1.71 (1.30)
C: Uninoculation control	22.50 (4.74)	15.50 (3.93)	3.90 (1.97)	5.78 (2.40)	2.47 (1.57)
S.E. ±	0.29	0.14	0.05	0.15	0.03
C.D. (P=0.05)	0.86	0.41	1.14	0.44	0.08
C.V. (%)	4.21	2.93	2.85	7.44	3.19

Figures in the parenthesis are square root transformed data

Root-knot index (5) as well as number of galls per plant (178.75) and number of egg masses per root (132.25) were maximum in plants inoculated with nematode alone (Table 2). Nematode inoculation followed by bacterium recorded 104.25 galls per plant, 83.50 egg masses per root system and root knot index 5. Plants simultaneously inoculated with nematode and bacterium recorded 75.75 galls per plant, 66.25 egg masses per root system with root knot index of 4. This might be due to the prior establishment of bacterium in roots which was not favourable for nematode multiplication. has been suggested that the bacterium modifies extensively host tissue which doesn't favour nematode multiplication. The present research work has clearly established that presence of nematode in the soil aggravates bacterial wilt as it is known to cause minute wounds serving as avenue for the entry of the vascular wilt pathogens. Swain (1987) also reported that the combined pathogenic effects of *Meloidogyne incognita* and *Ralstonia solanacearum* on a resistant brinjal cultivar (Pusa purple cluster) provided synergistic effect towards the development of wilt symptoms and affected different plant growth parameters such as shoot length, shoot weight, root length and root weight.

Significant reduction in plant height, fresh shoot weight, dry shoot weight, fresh root weight and dry root weight was noticed in all the treatments in comparison to uninoculated control (Table 3).

When the bacterium inoculated alone (B), plant height (15.8 cm), fresh shoot weight (10.71 g), dry shoot weight (4.20 g), fresh root weight (4.62) and dry root weight (1.85) was observed. Whereas in case of bacterial inoculation followed by nematode after 15 days (Bn), plant height (10.40 cm), fresh shoot weight (7.97 g), dry shoot weight (3.22 g), fresh root weight (3.89 g) and dry root weight (1.49 g) was observed. The adverse effect produced on plant height, fresh shoot weight, dry shoot weight, fresh root weight and dry root weight by bacterium alone (B) as well as inoculation with nematode after bacterial establishment (Bn) was statistically on par (Table 3).

When the nematode was inoculated followed by bacterium 15 days later, there was a significant reduction in plant mean height, weight of shoot and root. The plant height (8.40 g), fresh shoot weight (6.22 g), dry shoot weight (2.44 g), fresh root weight (2.58 g) and dry root weight (1.06 g) differed significantly from all other treatments (Table 3).

Similar findings were also reported by Napier and Quimo (1980) and Sitaramaiah and Sinha (1984) in tomato and brinjal, respectively when associated with bacteria. The data obtained in the present investigation clearly indicated that *M. incognita* played a significant role as a predisposing factor and thus increasing the incidence of wilt. Plants which received *M. incognita* and *R. solanacearum* separately as well as in three different combinations, thus recorded significantly lower plant height, fresh and dry shoot weight and fresh and dry root weight compared to healthy plants. The nematode inoculation followed by bacterium after 15 days (Nb) resulted in significantly lesser plant growth when compared to other treatments that received these inoculations.

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