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Interaction of *Ralstonia solanacearum* and *Meloidogyne incognita* on Tomato (*Solanumlyco persicon* L.)

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A B S T R A C T

The combined pathogenic effects of *Meloidogyne incognita* and *Ralstonia solanacearum* on tomato were greater than independent effects of either. Inoculation of *M. incognita*, 10 days prior to the inoculation of *R. solanacearum* led to maximum wilt incidence (100%) followed by combined inoculation of both the pathogens (66.66%). Low wilt incidence was noticed in plants inoculated with bacterium alone and which was on par with inoculation of bacterium10 days before inoculation of nematode.

Introduction

Tomato (*Solanum lycopersicon* L.; 2n = 24) belong to the solanaceae family, native to the Mexico. Tomato is a popular and widely grown vegetable in the world. In India, it occupies an area of 882 thousand hectares with a production of 18,736 thousand million tonnes and productivity of 21.24 metric tonnes per hectare (Anon., 2014).

In Karnataka, it is grown in an area of 61.04 thousand hectares, with a production of 2068 thousand million tonnes, with an average yield of 33.90 tonnes per hectare (Anon., 2014).Tomato is considered as an important source of dietary antioxidants, components such as lycopene, phenols, flavonoids and vitamins C are responsible for the antioxidant activity of tomatoes and processed tomato products. Bacterial wilt of tomato caused by *Ralstonia solanacearum* is prominent diseases and in extreme cases loss in yield due to this disease in eggplant and tomato has been reported to be as high as 80 and 90 per cent, respectively (Rao *et al.*, 1975). It has been proved that root knot nematode facilitates entry and establishment of pathogenic fungi and bacteria (Powell, 1971).

In recent years, the disease complex due to *M. incognita* and *R. solanacearum* has been gaining economic importance in tomato cultivation (Ravichandra *et al.*, 1990).

Material and Methods

Red sandy loam soil free of lumps and stones was mixed thoroughly with 1:1:1 proportion of soil, sand and FYM. The mixture was autoclaved at 1.05cm^2 pressure for two hours on two successive days. The sterilized soil was allowed to cool at room temperature and later used to fill the pots for various experiments.

Root-knot infected tomato plants were collected from the sick plot of AICRP (Nematodes), ZARS, GKVK, Bengaluru in polythene bags and kept in the freezer. Root portion was carefully removed from the soil and washed gently under running tap water. Egg masses were picked and kept for hatching in water in a Petri dish. After 24 to 36 h, juveniles hatched, which were used to inoculate twenty-one days old tomato seedlings grown in sterilized soil and maintained in glasshouse. Soil samples of 250g were washed thoroughly and processed using combined Cobb's and sieving and Baermann's funnel method (Ayoub, 1977) as described below.

250g of soil was taken in 1000ml beaker and sufficient quantity of water was added to make a soil solution

This was stirred thoroughly and allowed to stand for the heavier particles to settle down.

Then the soil solution was passed thoroughly through a set of sieves of 100, 250,325 and 400 mesh sizes

Residue from 325 and 400 mesh sieves were collected and poured over a tissue paper kept on the Baermann's funnel.

The level of water in the Baermann's funnel was maintained to keep the tissue paper wet and kept undisturbed for 48 h.

After 48 h of inoculation, the volume of suspension was made to 250 ml, out of which 10ml was pipetted out and used for counting of root-knot nematodes.

The galled roots were immersed in a beaker containing boiling solution comprising of 0.1 per cent cotton blue/lacto phenol for two minutes, cooled by washing in running tap water and again plugged into lacto phenol and left overnight for clearing (Goodey *et al.,* 1965). The root galls were dissected under the stereo binocular microscope with the help of sharp needles to release the female nematodes, which were then transferred to a drop of lacto phenol taken on a glass slide, the posterior portions of the females were carefully cut with a sharp blade under stereo binocular microscope. The inner material of the nematode was cleaned out with a nylon bristle and then trimmed and mounted for observation under oil immersion lens to identify the species of *Meloidogyne* on the perineal patterns. The identification of the species as *M. incognita* was confirmed on the basis of the perineal pattern described by Goodey *et al.,* (1965).

The egg masses from stock culture were transferred carefully on to a wire gauze sieve containing two layers of facial tissue paper trimmed down to edge of the gauze and kept in a Petri dish holding sufficient water to remain in contact with the bottom of the wire gauze and wet the egg masses. The hatched juveniles passed through the paper tissue and sunk to the bottom of the Petri dish. After 24 h, the contents of the Petri dish were emptied into a beaker, diluted to a suitable volume and population counts made with the help of a Fenwick's multi-chamber counting slide. Based on the requirement, the suspension was diluted with sterile water. Two weeks after transplanting tomato seedling cv. Arka Vikas in the test pots, the suspension containing a pre-determined number of juveniles was

pippeted uniformly over the surface of the roots which were carefully exposed earlier and then covering roots with soil and the plants were watered to keep the soil

One hundred microliter of the diluted bacterial suspension was poured onto the surface of the solidified triphenyltetrazollium chloride agar medium (Kelman, 1954) in sterilized Petri plates. The bacterial suspension was spread onto the, surface of TTC medium with a sterilized spreader. The inoculated plates were incubated at 32 °C for 48 h. At the end of the incubation period, the plates were observed for the development of well-separated, irregular, fluidal, dull white colonies with smooth red center typically of virulent *Ralstonia solanacearum* colonies. *Meloidogyne incognita* and *Ralstonia solanacearum* previously isolated from infected tomato plants were inoculated to the pots containing seedlings according to the treatment fixed. Inoculation was carried out by pouring around the base of each plant 5 ml of a suspension of 600 individuals per ml in sterile distilled water *i.e*. 3000 juveniles (J_2) per plant and the bacterial inoculum potential was spectrophotometrically adjusted to OD 600 nm = 0.1 (approximately 10^8 CFU/ ml). Inoculation was carried out by pouring 5 ml of bacterial suspension around the base of each plant.

Results and Discussion

Maximum per cent wilt incidence (100%) was recorded in plants inoculated with nematode prior to bacterial inoculation (Nb) followed by simultaneous inoculation of nematode and bacterium wherein 66.66 per cent of the plants were wilted at 45 days after inoculation (Table 1). Treatment with bacterium alone or bacterium inoculated prior to nematode inoculation showed slight difference in wilt incidence. Similar results were reported by Routaray *et al.,* (1986) who have found that inoculation of *M. incognita* seven days prior to *P. solanacearum* at planting caused maximum wilt (75%) followed by simultaneous inoculations of both the pathogens (45%) at 60 days of observation. No wilt was noticed in plants inoculated with nematode alone and inoculated plant showed very poor growth and chlorotic appearance.

The results were also in conformity with the work done by Haider *et al.,* (1989) who have reported that inoculation of *M. incognita* at 10 days prior to inoculation of *R. solanacearum* produced maximum wilt (100%) followed by simultaneous inoculation of both the pathogens (80% at 60 days). Minimum wilt was noticed with bacterium alone which was on par with bacterium inoculated before the nematode.

Treatment details

Treatments No.	Treatment details
T_1	Inoculation of *M. incognita* only (3000 J_2/ pot)
T_2	Inoculation *R. solanacearum* only (10^8cfu/ml)
T_3	Simultaneous inoculation of *M. incognita* (3000 J_2/ pot) and *R. solanacearum* (10^8cfu/ml)
T_4	Inoculation of *M. incognita* (3000 J_2/ pot)10 days before inoculation of *R. solanacearum* (10^8cfu/ml)
T_5	Inoculation of *R. solanacearum* (10^8cfu/ml)10 days before inoculation of *M. incognita* (3000 J_2/ pot)
T_6	Control

Table.1 Effect of sequential inoculation of *Meloidogyne incognita* and *Ralstonia solanacearum* on the incidence of bacterial wilt on tomato

Treatments	Wilt incidence (%)		
	Intervals of observation		
	25 Days	35 Days	45 Days
T_1: Inoculation of *M. incognita* only (3000 J_2 / pot)	0.00	0.00	0.00
T_2: Inoculation *R. solanacearum* only (10^8cfu / ml)	0.00	0.00	33.33
T_3: Simultaneous inoculation of *M. incognita* (3000 J_2 / pot) and *R. solanacearum* (10^8cfu / ml)	0.00	33.33	66.66
T_4: Inoculation of *M. incognita* (3000 J_2 / pot) 10 days before inoculation of *R. solanacearum* (10^8cfu / ml)	33.33	66.66	100
T_5: Inoculation of *R. solanacearum* (10^8cfu / ml)10 days before inoculation of *M. incognita* (3000 J_2 / pot)	0.00	0.00	33.33
T_6: Control	0.00	0.00	0.00

Table.2 Effect of sequential inoculation of *Meloidogyne incognita* and *Ralstonia solanacearum* on growth parameters of tomato plants

Treatments	Plant height (cm)	Shoot weight (g)		Root weight (g)	
		Fresh	Dry	Fresh	Dry
T_1: Inoculation of *M. incognita* only (3000 J_2 / pot)	30.83^{b}	14.31^{b}	5.41^{b}	2.03^{b}	0.81^{ab}
T_2: Inoculation *R. solanacearum* only (10^8cfu / ml)	25.23^{c}	12.03^{c}	4.41^{c}	1.65^{c}	0.65^{bc}
T_3: Simultaneous inoculation of *M. incognita* (3000 J_2 / pot) and *R. solanacearum* (10^8cfu / ml)	21.33^{e}	10.79^{d}	3.96^{d}	1.47^{e}	0.58^{bc}
T_4: Inoculation of *M. incognita* (3000 J_2 / pot)10 days before inoculation of *R. solanacearum* (10^8cfu / ml)	15.30^{f}	6.11^{e}	2.23^{e}	0.82^{f}	0.32^{c}
T_5: Inoculation of *R. solanacearum* (10^8cfu / ml)10 days before inoculation of *M. incognita* (3000 J_2 / pot)	24.83^{d}	12.02^{c}	4.41^{c}	1.61^{d}	0.63^{bc}
T_6: Control	48.70^{a}	26.23^{a}	9.92^{a}	3.74^{a}	1.48^{a}
S. Em ±	0.10	0.02	0.01	0.01	0.01
CD @ 5%	0.32	0.06	0.03	0.03	0.03

Table.3 Effect of sequential inoculation of *Meloidogyne incognita* and *Ralstonia solanacearum* on development of root-knot nematode on tomato plants

Treatments	No. of galls per root system	No. of egg masses per root system	Root-knot index (0-5 Scale)
T_1: Inoculation of *M. incognita* only (3000 J_2 / pot)	124.67^{a} (11.18)	62.00^{a} (7.90)	5
T_2: Inoculation *R. solanacearum* only (10^8cfu / ml)	0.00^{e} (0.70)	0.00^{e} (0.70)	0
T_3: Simultaneous inoculation of *M. incognita* (3000 J_2 / pot) and *R. solanacearum* (10^8cfu / ml)	30.00^{c} (5.51)	15.67^{c} (4.01)	3
T_4: Inoculation of *M. incognita* (3000 J_2 / pot) 10 days before inoculation of *R. solanacearum* (10^8cfu / ml)	83.33^{b} (9.15)	41.33^{b} (6.46)	4
T_5: Inoculation of *R. solanacearum* (10^8cfu / ml)10 days before inoculation of *M. incognita* (3000 J_2 / pot)	10.00^{d} (3.23)	5.67^{d} (2.46)	2
T_6: Control	0.00^{e} (0.70)	0.00^{e} (0.70)	0
S. Em ±	2.38 (0.073)	1.76 (0.08)	-
CD @ 5%	7.32 (0.48)	5.43 (0.50)	-

Figures in the parentheses are square root transformed values

The data obtained in the present investigation clearly indicated that *M. incognita* plays a significant role as a predisposing factor by making the punctures on roots which leads to easy invasion of pathogen and thus increasing the incidence of wilt. Plants which inoculated with *M. incognita*10 days prior to inoculation of *R. solanacearum* recorded significantly lower plant height (15.30 cm), fresh and dry weight shoot (6.11 and 2.23 g) and root (0.82 and 0.32 g)compared to control (Table 2). This might be due to inoculation of nematode before bacterium retards the growth of plant by forming galls on roots which distracts the flow of nutrient and water throughout the plant leading to the reduction in growth parameters. Further, Swain (1989) also reported that the combined pathogenic effects of *M. incognita* and *R. 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.

Number of galls (124.67), number of egg masses (62) and root-knot index (5) were maximum for nematode treatment alone but it was significantly less in other treatments (Table 3). Perhaps prior establishment of bacterial colonies in the rhizosphere was not conducive for nematode multiplication. Pitcher (1963) has suggested that the bacterium modifies extensively host tissue which does not favors nematode multiplication. This could be the reason for decreased nematode multiplication in the present study.

References

Anonymous, 2014, National horticulture board, 2013-14 database.

Ayoub, S. M., 1977, Plant Nematology- An agricultural training aid. State of California, *Dept. of food and Agric., Sacromento,* 156 pp.

Goodey, J. B., Franklin, M. T. and Hoper, D. J., 1965, the nematode parasites of plants catalogued under their hosts. *Common welth Agric. Bur., U.,* 214 pp.

Haider, M. G., Nath, R. P., Thakur, S. C. and Ojha, K. L., 1989, Interaction of *Meloidogyne incognita* and *Pseudomonas solanacearum* on tomato plants. *Indian J. Nemotol.*, 17: 174-176.

Kelman, A., 1954, the relationship of pathogenicity of *Pseudomonas solanacearum* to colony appearance in tetrazolium medium. *Phytopathol,* 44: 693-695.

Pitcher, R. S., 1963, Role of plant parasitic nematodes in bacterial diseases. *Phytopathol*, 53: 35-39.

Powell, N. T., 1971, Interaction of plant parasitic nematodes withother disease causing agents. In: Plant parasitic nematodes. *Academic press*. 2: 99-136.

Rao, M. V. B., Sohi, H. S. and Tirao, S. K., 1975, Reaction of wilt resistance tomato varieties and lines to *Pseudomonas solanacearum* in India. *Pl. Dis. Reptr*., 59: 734-736.

Ravichandra, N. G., Krishnappa, K. and Setty, K. G. H., 1990, Interaction of *Meloidogyne javanica* and race-1, race-2, race-3 of *M. incognita* with *Pseudomonas solanacearum*on a few brinjal cultivar/lines. *Indian J. Nematol*., 20(2): 138-147.

Routaray, N., Sahoo, H. and Das, S. N., 1986, Response of some nematode tomato cultivars to inoculations of *Meloidogyne incognita* and *Pseudomonas solanacearum*. *Indian J. Nematol*., 16: 56-59.

Swain, P. K., Rath, J. C. and Mishra, S. K., 1989, Interaction between *Meloidogyne incognita* and *Pseudomonas solanacearum* on brinjal. *Indian J. Nematol*., 17: 67-71.

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