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Journal of Plant Pathology & Microbiology

Research Article

Effect of Combined Inoculation of *Xanthomonas* and *Meloidogyne* Pathogens on the Development of Tomato Root Knot Disease

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ABSTRACT

Bacteria (*Xanthomonas campestris* pv. *vesicatoria*) and root-knot nematode (*Meloidogyne incognita*) independently can damage and cause considerable damage to tomato (*Solanum lycopersicum* L.). In a disease complex, interrelationship of 2 or more pathogenic species can produce different symptoms on the same host plant. Generally simultaneous occurrence of these pathogens in a field can infect hosts plant at the same time. During development of a disease complex pathogens could influence and/or suppress each other, through synergism and or antagonism respectively. In this study the pathogens (*Meloidogyne incognita* and *Xanthomonas campestris* pv. *vesicatoria*), were used to determine how co-occurrence affects development of pathogens and disease severity, and define prerequisites for interrelation between pathogens.

Root knot infection did not occur when tomato plants were inoculated with *Xanthomonas campestris* 1 week prior to inoculation with *Meloidogyne incognita*. When *M. incognita* was inoculated 1 week prior to *X. campestris*, infection by root knot nematode was highest compared to bacterial spot incidence in susceptible plants. Simultaneous inoculation of *M. incognita* + *X. campestris* caused severe gall production with moderate severity of bacterial spot disease. The reproduction of 1 pathogen can be affected by a subsequent inoculation of other pathogen. It is suggested that bacterial spot disease enhances the development of root knot disease.

Keywords: *Solanum lycopercicum*; Bacterial spot; Disease complex; Simultaneous

INTRODUCTION

Tomato (*Solanum lycopersicum* L.) has high nutritive value and is consider as a protective food. Tomato plants can be exposed to a wide range of abiotic and biotic challenges that cause yield loss. Resistance and susceptibility to these challenges are complex, because at multiple stages of plant development detrimental effects may occur. Generally, more than 1 challenge simultaneously affects the plant. The tropical environment can be favorable for occurrence of many pathogens and a disease complex can occur on the same host. The study of the interaction may be important because expected benefits from control of 1 pathogen may depend on the level of other pathogens. Among causal organism of disease the nematode (*Meloidogyne incognita*) contributes to spread of a broader disease [1]. In a nematode- bacterial disease complex bacteria can enter host tissue via root wounds caused by root knot nematode and the bacteria can move to xylem vessels where they multiply and spread [2,3]. Nematodes cause direct and indirect damage through reduced water uptake and nutrients cannot be taken up through damaged roots [4].

X. campestris pv. *Vesicatoria* (*Doidge*) causes the bacterial spot disease of tomato (*Lycopersicon esculentum*) and Pepper (*Capsicum annum*) plants. Necrotic spots on foliage and fruit water soaked lesions are characteristics of the disease. *X. campestris* is extensive and harmful to chilli and tomatoes in the crops that are grown in fields at warmer areas where having overhead irrigation to field. *X. campestris* secretes different products which contributing in pathogenicity. Such as exo-polysaccharide, Pectolytic, Proteolytic and cellulolytic enzymes and toxic metabolites.

These pathogens, including *Xanthomonas*, are often associated

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Received: June 09, 2020; **Accepted:** August 14, 2020; **Published:** August 21, 2020

Citation: Arain MR, Aslam MN, Jabeen S, Akram S, Shakeel Q, Sarwar N, et al. (2020) Effect of Combined Inoculation of Xanthomonas and Meloidogyne Pathogens on the Development of Tomato Root Knot Disease. J Plant Pathol Microbiol. 11: 510. doi: 10.35248/ 2157-7471.20.11.510

with roots of tomato under field conditions. Usually, a soil-borne nematode modifies plant response to other pathogens at the time of inoculation. Nematodes modify the host plant physiology by causing injury. Infected plants are generally small sized and extensive gall development, wilt symptoms and leaves spot. In disease complexes including nematodes, particularly the sedentary endo-parasitic root knot nematode M. *incognita* and the bacterium *Ralstonia solanacearum* the severity of bacterial wilt [2,5-9] were highest. This study was undertaken to determine combined effects of pathogens on plant growth and how pathogens interact with each other. The main objective to conduct this study was that interaction of both pathogens increase the severity of root knot nematode (RKN) disease. The study was undertaken to determine how order of inoculation of pathogens affected disease development and tomato root growth.

MATERIAL AND METHODS

The experiments were performed in a greenhouse at the Government Shrub Nursery, Bahawalpur, Pakistan in 2018-2019. Fifty percent river soil, 20% peat moss, and 30% sandy loam were mixed and used as the growing medium at temperature of 30-35°C and 61% humidity. The soil was exposed to in sunlight for 15-20 days and placed in 1 kg clay pots. Seed were surface sterilized for 2 min in 0.1% sodium hypochlorite solution and washed 3 times with purified water. Seed were sown in the growing medium in pots. Twenty-one days old seedlings of genotypes Rio Grande, Raffal, Bss-30 and Hybrid 2017 were thinned to 3 seedlings per pot. Seedlings were provided with 100 mL/pots of water daily throughout the experiment.

Pots were arranged in a completely randomized design. During the present study 05 treatments were used viz; *Meloidogyne incognita (Mi), X. campestris (X.c),* M. *incognita first* and X. *campestris later* (*Mi* → *XC), X. campestris* first and M. *incognita* later (X.C → M.i), Simultaneous inoculation (M.i +X.C), C (control).

Five un-inoculated pots / 3 plants served as the control. Two days after thinning of seedlings treatments were applied. Three plants per each treatment were used for the determination of bacterial and root knot nematode symptoms and morphology. The infected leaves showing the typical symptoms of bacterial spot were collected from tomato plants which grown in green house of Department of Plant Pathology, The Islamia University of Bahawalpur, Pakistan. In laboratory ooze test is performed by cutting 1cm of the infected portion of plant, sterilized gentely squeezed to get a suspension in a sterile test tube containing 3 ml of sterilized saline. After diluted it is plated on YDC Agar medium. After 24 hrs incubation light yellow color colonies were developed resembling *Xanthomonas* sp. biochemical assay were carried out for the confirmation of X. *campestris* species. Recovered colonies were harvested and suspended in flasks containing sterilized distilled water and incubated for 2-4 h at 32 ± 1°C. Inoculum potential was adjusted with a spectrophotometer. Nematode inoculum of M. *incognita* was obtained from soil of an infested field and affected plant roots. Inoculum of root knot nematode was prepared following methods of Whitehead and Hemming [10]. Shoots of infested plants were detached, and roots carefully washed to detach soil particles. Large numbers of egg masses of *Meloidogyne* species were recovered from roots of infected host plants. Egg masses were placed on a double layer of tissue paper supported by a coarse plastic net placed over a plastic tray full of water. After 48 h the net with tissue paper was removed and the water was collected in a beaker and left for about 2-3 h to get settled. Nematodes were recovered and used as the inoculum. Two mL of stirred suspension was placed in a counting dish to determine the population of second stage juveniles (J2) of root-knot nematode using a stereoscopic microscope. An average of 3 counts was used to determine nematode density. The volume of water was adjusted so that 1 mL contained about 100 nematodes.

Roots of plants in pots were uncovered and the nematode suspension (1000 j2 /plant) poured on the roots which were recovered with the growing medium. While ten milliliters (107 CFU mL-1) of bacterial suspension was inoculated into each pot around the tomato seedlings. In control plants an equal amount of sterile water was added. After 45 days plants were harvested. applied.

The genotypes were scored according to the root-knot rating scale of Taylor and Sesser [11] and rating scale of root-knot infestation in tomato under greenhouse conditions. The method of estimation of bacterial spot index was a 0-5 scale where 0 = no disease and 5 = severe spot index. The method of estimation of disease severity was according to Wai et al. [12]. The 5 category scales were reduced to 4 by merging categories 3 and 4 of the original scale. The categories of altered scale were 1 = no visible symptoms, 2 = one leaf to half of the foliage wilting, 3 = nearly all of the foliage wilting, 4 = the whole plant dead. Disease severity (%) for each treatment was computed using the formula of Bdliya and Dahiru. After 45 days following inoculation plants were harvested and data of plant length (shoot length + root length), number of branches, number of leaves, shoot and root fresh weights, and shoot and root dry weight were determined.

Data of root knot reproduction factors were log transformed (log (y+1)). Data were subjected to analysis of variance by using online Bio-stat-2010 software. If the interaction was significant it was used to explain the results. if the interaction was not significant the means were separated using Fisher's LSD.

RESULTS

Effect of Alone *M. incognita*

In the present study two varieties Super special and Hybrid 2017 were highly resistant and susceptible varieties. The highest damage index (DI) was observed in Early King and Rio Grande. While gall index and egg mass index showed that reproduction of M. incognita is favoured on susceptible varieties. The highest damage index i.e., > 1 are observed in Rio Grande and Early King. Highest mean population of second stage juveniles (4335) was recorded from Early King variety followed by Rio Grande (2295). While the least population was recorded from Hybrid-2017 and Super special i.e., 95 and 67 J2 respectively (Figure 1).

Incidence and severity of *X. campestris* disease

The observations showed that the highest (41.66%) bacterial leaf spot incidence was recorded in Raffal while in case of moderately susceptible variety i.e., BSS-30 (33.3%). Moreover the least (20%) bacterial leaf spot incidences was measured in Hybrid-2017 (Figure 2).

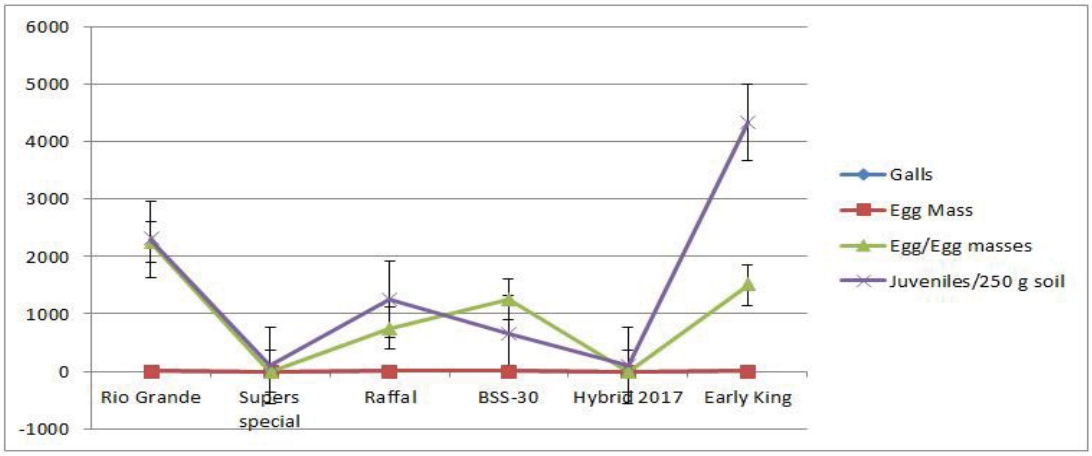

Figure 1: *M. incognita* reproduction in tomato varieties.

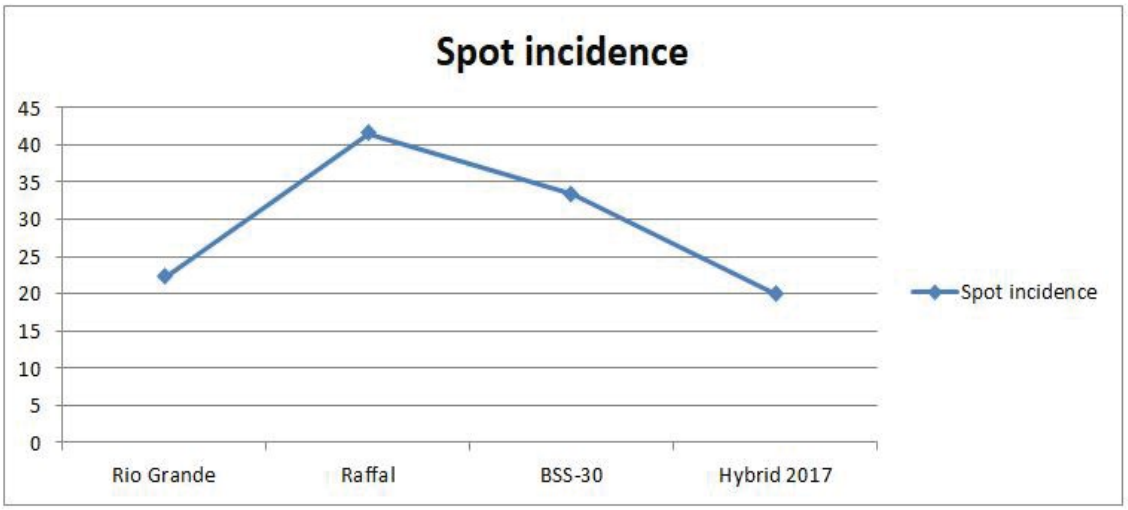

Figure 2: Spot incidence in tomato varieties.

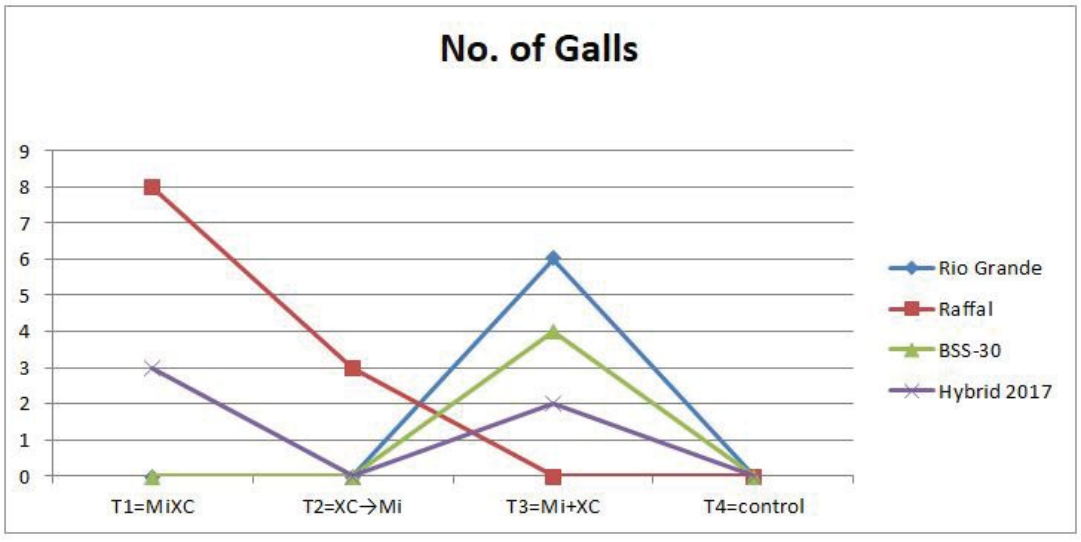

Figure 3: Number of galls in tomato varieties.

Simultaneous inoculation and development of RKN disease:

In case of simultaneous inoculations when M. *incognita* inoculated 1 week prior to *Xanthomonas campestris*, susceptible plants exhibited highest root knot nematode infection compared to spot incidence. Maximum number of galls were recovered, and similarly highest spot incidence was recorded in both susceptible varieties (Figure 3). Galls were not produced in remaining tested varieties that showed low incidence of bacterial spot. When we proceeded with X. *campestris* before inoculation of M. *incognita* unexpected and opposite results were observed. Medium size galls were observed

Table 1: Synergistic effect of Xanthomonas campestris and Meloidogyne incognita on growth parameters of tomato plant.

Varieties	Treatments	Plant length (cm)	Root length (cm)	Fresh shoot weight (g)	Fresh root weight (g)	Dry shoot weight (g)	Dry root weight (g)
Rio Grande	T1	21.1^{b}	9.1^{c}	1.46^{b}	0.33^{b}	0.30^{a}	0.09^{b}
	T2	19.7^{bc}	5.2^{e}	1.10^{b}	0.10^{c}	0.22^{b}	0.03^{c}
	T3	18.2^{bc}	6.9^{de}	2.71^{a}	0.46^{b}	0.22^{b}	0.02^{c}
	T4	10.4^{d}	3.4^{f}	0.29^{e}	0.05^{e}	0.08^{e}	0.02^{c}
	T5	33.6^{a}	16.7^{a}	1.01^{b}	0.11^{c}	0.9^{d}	0.016^{d}
	T6	24.7^{b}	6.5^{de}	1.12^{b}	0.12^{c}	0.28^{b}	0.04^{c}
Raffal	T1	18.8^{b}	8.5^{c}	0.69^{c}	0.18^{c}	0.16^{c}	0.05^{b}
	T2	17.2^{bc}	8.5^{c}	0.41^{d}	0.08^{d}	0.10^{d}	0.03^{c}
	T3	18.2^{bc}	6.9^{de}	0.81^{c}	0.30^{b}	0.14^{c}	0.05^{b}
	T4	15.1^{c}	5.3^{e}	0.42^{d}	0.26^{bc}	0.21^{b}	0.03^{c}
	T5	15.1^{c}	5.3^{e}	0.82^{c}	0.15^{c}	0.20^{b}	0.08^{b}
	T6	16.7^{c}	7.6^{d}	0.85^{c}	0.08^{d}	0.16^{c}	0.04^{c}
BSS-30	T1	30.7^{a}	17.2^{a}	2.12^{a}	0.71^{a}	0.45^{a}	0.23^{a}
	T2	24.8^{b}	15.5^{a}	0.74^{c}	0.14^{c}	0.24^{b}	0.06^{b}
	T3	31.7^{a}	16.6^{a}	0.60^{d}	0.11^{c}	0.14^{c}	0.03^{c}
	T4	13.7^{c}	6.2^{de}	0.39^{e}	0.07^{e}	0.08^{e}	0.02^{c}
	T5	31.7^{a}	16.^{8a}	2.05^{a}	0.24^{bc}	0.40^{a}	0.08^{b}
	T6	15.2^{c}	6.1^{de}	0.59^{d}	0.09^{d}	0.13^{c}	0.03^{c}
Hybrid 2017	T1	21.7^{b}	7.4^{d}	0.95^{bc}	0.74^{a}	0.22^{b}	0.07^{b}
	T2	13.3^{c}	5.6^{e}	0.44^{d}	0.08^{d}	0.10^{d}	0.02^{c}
	T3	28.3^{a}	14.1^{b}	1.85^{b}	0.32^{b}	0.31^{a}	0.07^{b}
	T4	31.2^{a}	16.2^{a}	3.52^{a}	0.29^{b}	0.76^{a}	0.10^{a}
	T5	18.4^{bc}	7.4^{d}	0.98^{c}	0.16^{c}	0.22^{b}	0.05^{b}
	T6	25.4^{a}	14.8^{b}	0.85^{c}	0.10^{c}	0.17^{c}	0.03^{c}
Treatment × Varieties		0.0067		0	0	0.5374	0.0549

T1 = Alone M. *incog*, T2 = Alone *Xanthomonas*, T3 = RKN→X.c, T4 = X.c → RKN, T5 = RKN + X. c, T6 = Without inoculation.

*in a columns small letter exhibit are non-significantly different at P < 0.05 LSD.

with 26.9% spot incidence in Raffal. While galls were not produced in other tested varieties but spot severity occurred in varying range. High incidence was recorded in Rio Grande variety. Simultaneous inoculation of *Meloidogyne incognita* and *Xanthomonas campestris* to caused severe galls production with moderate severity of bacterial spot disease. In case of simultaneous inoculation, when root knot nematode disease incidence was high, than low incidence of spot disease was occurred. Root knot nematode reproduction and galls were not observed in which varieties were exhibited moderate to highest incidence of the *Xanthomonas campestris*. Inoculation with *Xanthomonas campestris* prior to *Meloidogyne incognita* were caused greater reduction in galling and nematode multiplication compared to inoculation with together or with *Meloidogyne incognita* prior *Xanthomonas campestris*. The bacterial spot incidence was 50% in case of Xanthomonas campestris was introduced before *Meloidogyne incognita*. 21% incidence was observed when *Meloidogyne incognita* was introduced before *Xanthomonas campestris* and 36.3% incidence was noted in simultaneous inoculation of both pathogens.

Plant growth

Inoculation with M. incognita prior to X. *campestris* was increased plant growth in all tested varieties contrast to plants which inoculated with X. *campestris* prior to M. *incognita* except Hybrid 2017 variety. Inoculation with both pathogens together were exhibited maximum plant growth in Rio Grande and BSS-30 varieties. In Raffal and Hybrid 2017 highest plant growth reduction was recorded than control plants.

Root length was significantly reduced when plants were inoculated with X. *campestris* prior to M. *incognita* except Hybrid 2017 (Table 1). Inoculation with M. *incognita* before X. *campestris* caused a reduced root length in Rio Grande and Raffal varieties. Susceptible varieties viz., BSS-30 and Hybrid exhibited maximum primary root length without rootlets or secondary root system (Figure 4). Similarly highest primary root length was observed in Rio Grande and BSS-30 when both pathogens introduced simultaneously compared to control plants. M. *incognita* had adverse effect with X. *campestris* on root length, by producing long and thin primary roots without normal root system. Significant results were observed in fresh shoot

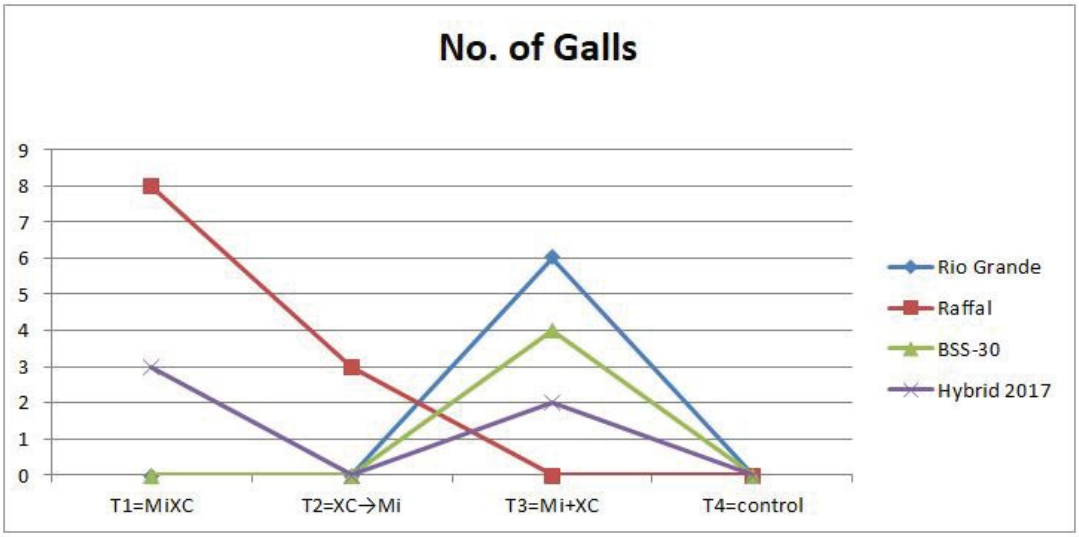

Figure 4: significantly reduction of root length.

weight. Significantly reduction in fresh shoot weight was measured in treatment 2 when plants inoculated with *X. campestris* prior to *M. incognita* except Hybrid-2017 variety which exhibited maximum fresh shoot weight than inoculated with *M. incognita* prior to *X. campestris*. In case of simultaneously inoculation with both pathogens together caused a reduction in all varieties except BSS-30 was observed than treatment 1 and 2. Reduced fresh root weight was observed when plants inoculated with *M. incognita* prior to *X. campestris*.

Maximum root weight was recorded when inoculated with *X. campestris* prior to *M. incognita*. Highest root weight is due to the presence of nematode galling and reproduction in roots. Rio Grande was highly susceptible with gall index was (4). In treatment 3 (simultaneous inoculation) fresh root weight was highly reduced. Reduced weight was produced by long and thin primary roots, lacking of rootlets and secondary roots (deformed structure of roots). Table 1 shows that reduced shoot weight was observed when plants inoculated with *M. incognita* prior to *X. campestris* and simultaneous inoculation. Rio Grande and BSS-30 exhibited maximum dry shoot weight while Raffal and Hybrid-2017 showed almost same dry shoot weight compared to control plants when inoculation with both pathogens together. Reduction in dry root weight was observed in Rio Grande in all treatments as compared to control. In Raffal and BSS-30 similar and maximum dry root weight was noted when simultaneously inoculation with both pathogens. Hybrid 2017 exhibited highest dry root weight when inoculation with *X. campestris* prior to *M. incognita* compared to other treatments.

DISCUSSION

Co-occurrence of root knot nematode with *X. campestris* cause disease complex in tomato plants. We investigated that in case of alone inoculation of each pathogens caused a considerable decline in plant growth over the control. Previous study of Caillaud et al., [13] reported that in *Meloidogyne - Xanthomonas* disease complex, nematodes induced feeding cell formation in plants by this fact fundamental elements of plant cell development also manipulate and cause loss of yield. In this study we observed similar findings. In the present study, we not observed root knot infection when plants inoculated with *X. campestris* prior to *M. incognita*. Bacterial spot disease characterized by the production of water-soaked lesions of young leaf tissue. However we observed that in later stage the infested tissues lose their structural integrity and disintegrate, taking on a soft rot appearance, due to these reasons long, thin and lateral roots were not produce so *M. incognita* feeding sites are not available. Previous studies confirmed that simultaneous inoculation causes severe disease symptoms in tomato plants. During plant disease complex both pathogens may have dual effects on disease severity i.e., direct and indirect. Plant parasitic nematodes cause physical damage by their stylet quickly that can allow secondary infection through other pathogens [14-16]. Similar findings were revealed by Siddiqui et al., [9,17], they revealed that wounds which caused by nematodes in favor of soil borne bacteria compared to fungi, because it is less active for penetration into host epidermis. In this study we observed that disease symptoms similar to those occurring in nematode-bacteria wilt interactions. Many previous studies such as Siqqiqui et al., [17] and Lucas et al., [18] confirmed this mechanism by conducting experiment in laboratory he mechanically injured chickpea roots by needle and plants inoculated with *X. campestris*. In the present study we monitored moderate to severe symptoms of bacterial spot when *Xanthomonas* bacteria inoculated prior to *Meloidogyne* nematode. However we don't observed bacterial spot symptoms when *M. incognita* inoculated prior to *X. campestris*. Similar findings were observed by Lucas et al., [18], and Siddiqui et al., [9], revealed that *X. campestris* cause adverse effects on the reproduction of *M. incognita* due to competition for the same host substrate. Previous studies revealed that the unfavorable effect of bacteria on nematodes caused by the secretion of bacterial toxin which inhibit the root galling and reproduction of root knot nematodes [7,14], they also justify these results that bacteria induced changes in the plant defense system which are not favorable for nematode reproduction. In this study we examined that plant was less infected in case of pre inoculation of bacteria contrasted to simultaneous inoculations. Our present findings corroborate with observations made by Hussain and Bora [7], Bhagwati et al., [19] and Hazrika [20], they noted significantly poor galls and egg masses in jute when *M. incognita* was associated with *Ralstonia solanacearum*.

The present investigations clearly explain the significant

variation in the plant growth parameters due to interaction effects of pathogens. In this study we assessed reduction in plant growth when prior inoculated with *X. campestris* < simultaneous inoculation < *M. incognita*. The present findings surely confirms the observation made by Sitaramaiah and Sinha [21] and Ghosh and Dutta [22], they were stated that increased reduction in plant heights in combined interaction of *M. incognita* with *R. solanacerum* in brinjal plant.

CONCLUSION

According to the findings of this study we have got a conclusion that the effects are most severe when *Meloidogyne incognita* is inoculated prior to the inoculation of *Xanthomonas campestris*. The effect was less severe in case when *Meloidogyne incognita* and *Xanthomonas campestris* were inoculated separately. These findings confirmed that root knot nematodes puncture the cell and change plant physiology for secondary pathogen entrance.

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