



Interaction between *Meloidogyne javanica* and *Ralstonia solanacearum* in Chili

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ABSTRACT

The information on the interaction between root-knot nematodes and bacterial wilt is lacking in chili, therefore, in the present study, effects of *Meloidogyne javanica* and *Ralstonia solanacearum* were studied on chili singly and in combinations. Significant effects of *M. javanica* and *R. solanacearum* and their interaction were observed in case of shoot and root lengths and weights of chili. The mean lengths and weights of shoot and root varied significantly as a result of both the pathogens applied singly and in combinations. The reductions in these parameters were significantly higher when both the pathogens were applied simultaneously as compared to their sole applications. Similarly, at higher inoculum densities, the reductions were the maximum in all these parameters. Significant variations were observed in number of galls and egg masses per root system in response to both the pathogens applied individually and in combinations. Maximum numbers of galls and egg masses were observed where nematode was applied alone. Significant reductions in galls and egg masses were recorded where nematode and the bacterium were applied in combinations. At higher densities of both the pathogens, the reductions in these parameters were the maximum. The first wilt appearance was observed after 8 days in the treatment where both the pathogens were applied at their highest densities. The treatments where bacterium was applied individually at different densities, wilt took maximum days to appear as compared to the treatments where bacterium and nematodes were applied in combinations. Similarly, the incidence of bacterial wilt was lower where the nematode was absent. Minimum wilt incidence was recorded at the lowest density of *R. solanacearum*. The wilt incidence increased significantly when nematode was inoculated along with the bacterium and maximum incidence was recorded where both the pathogens were applied at their highest densities.

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Authors' Contribution

AA, TM and MUR designed the study and executed the experimental work. TM and AG analyzed the data. TM, MUR and AG supervised the work and helped in preparation of the manuscript.

Key words

Root-knot nematode, bacterial wilt, incidence, interaction, Disease complex

INTRODUCTION

Different kinds of phytopathogenic bacteria including *Clavibacter michiganensis* subsp. *michiganensis*, *C. michiganensis* subsp. *insidiosus*, *C. michiganensis* subsp. *nebraskensis*, *Erwinia trachiphila*, *Pantoeaste wartii* are the causal organisms of bacterial wilt in different types of plants. Among all these, *Ralstonia solanacearum* inciting bacterial wilt disease has caused significant yield losses and has achieved an alarming position in chili growing areas. The low yield of chili is attributable to a number of biotic factors including insect pests (Javed *et al.*, 2017a, b, 2019; Aslam *et al.*, 2019a), fungi (Fateh *et al.*, 2017), viruses (Ashfaq *et al.*, 2017), nematodes (Kayani *et al.*, 2017, 2018; Khan *et al.*, 2017; Mukhtar *et al.*, 2017a; Mukhtar, 2018) and particularly the bacteria (Aslam *et al.*, 2019b).

R. solanacearum has wide geographic distribution and host range (Hayward, 1994) and infects more than

451 plant species from 54 botanical families (Wicker *et al.*, 2007). The pathogen invades xylem as vascular inhibiting bacteria. The pathogen is dispersed by different means viz. irrigation water, budding, grafting, ornamental tubers, potatoes, vegetables and banana (Flores-Cruz and Allen, 2009) but the most damaging source of dispersal is waterways (Elphinstone, 2005). The bacterium enters through natural openings and moves towards the xylem vessels where it blocks the supply of minerals and water and causes vascular browning, yellowing and stunting in many plants (Smith, 1920; Sarkar and Chaudhuri, 2015). Other symptoms include narrow dark strips beneath the epidermis, growth of adventitious roots on the stem, epinasty beneath the leaves, and oozing of bacteria in the form of slimy material when the infected stem is cut (Sarkar and Chaudhuri, 2015). The bacterium causes bacterial wilt which is a destructive disease of many plants and has caused significant yield losses in solanaceous crops worldwide (Yabuuchi *et al.*, 1995; Hayward *et al.*, 2005). In European Union it is considered as a quarantine pest and a potential bioterrorism agent in USA (Lambert, 2002).

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The first evidence of this disease in Pakistan was recorded during the year 1989 (Geddes, 1989). Later on Burney *et al.* (1999) investigated this pathogen in main solanaceous crops growing areas of Sindh and Punjab of Pakistan and recorded its incidence and severity. The pathogen spread with irrigation and rain water and moon soon (rainy season) played a significant role in the dispersal of this pathogen and chili is heavily attacked by this pathogen during this season. The attack was visible with wilting and caused heavy yield losses in chili crop.

The incidence and severity of bacterial wilt increased when root-knot nematodes were found present conjointly with *R. solanacearum* in many vegetables (Tariq-Khan *et al.*, 2017; Kayani and Mukhtar, 2018; Mukhtar *et al.*, 2018; Hussain and Mukhtar, 2019). It has been known for a long time that root-knot nematodes facilitate entry and establishment of pathogenic fungi and bacteria (Powell, 1971). In brinjal, the disease complex has been observed frequently involving *R. solanacearum* and root-knot nematode contributing to an increase in wilt development (Swain *et al.*, 1987).

The information on the interaction between root-knot nematodes and bacterial wilt is lacking in chili, therefore in the present study effects of *Meloidogyne javanica* and *R. solanacearum* were studied on chili singly and in combinations.

MATERIALS AND METHODS

Bacterial culture

A highly virulent isolate of *R. solanacearum* commonly prevalent in Pakistan was used in the studies. The bacterium was isolated from diseased plants showing typical bacterial wilt symptoms, confirmed immunologically and purified from a single colony. The hypersensitive reaction was performed on tobacco and tomato plants and pathogenicity was confirmed on susceptible cultivars of tomato and tobacco. After molecular confirmation, the purified culture of the highly virulent isolate was prepared and adjusted to 10^5 , 10^6 and 10^7 cfu/ml (Aslam *et al.*, 2017a, b).

Nematode inoculum

A purified culture of *M. javanica* initiated by a single egg mass and propagated on tomato cv. Money maker was used in the experiment. Twenty four hours old second stage juveniles (J2s) of the nematode were extracted by following the method described by Whitehead and Hemming (1965), standardized, concentrated and used for inoculation of chili plants.

Raising of chili nursery

The nursery of chili cv Bahawalpur Desi moderately resistant to bacterial wilt (Aslam *et al.*, 2017a) was raised in sterilized potting mixture in germination trays in the greenhouse. The daily temperature of the greenhouse ranged 25-27°C. The trays were watered when required.

Assessment of R. solanacearum and M. javanica on chili

The effects of *R. solanacearum* and *M. javanica* were assessed on chili in polythene bags measuring 12.75×10.15 cm. The bags were filled with sterilized soil containing 3:1:1 sand, silt and compost respectively. Three week old seedlings of chili cv Bahawalpur Desi were transferred individually to polythene bags. One week after transplantation, the plants were inoculated with *M. javanica* @ 0, 1000 and 2000 J2s per plant and *R. solanacearum* @ 0, 10^5 , 10^6 and 10^7 cfu/ml singly and in combinations. There were six replications for each treatment. The bags were arranged randomly in a glasshouse at a temperature of 25°C for six weeks and were properly moistened at alternative days.

Data collection

After inoculation, the wilt symptoms and wilted plants were recorded and graded with disease rating scale of 0-5 (Winstead and Kelman, 1952). The plants were gently removed from their respective pots after a period of six weeks. The shoots were excised from the roots and their lengths and weights were measured. The galls and egg masses of the whole root systems of plants were counted under stereoscope at magnification of 40×.

Statistical analysis

The experiment was conducted using completely randomized design. Percent reductions in growth parameters were calculated over controls prior to statistical analysis (Mukhtar *et al.*, 2017b). All the data were analyzed statistically employing GenStat software 12th edition (version 12.1.0.3278) and two way analysis of variance was performed. For comparison among means for significant differences, Fisher's protected least significant difference test was used at ($P \leq 0.05$).

RESULTS

Effect on growth parameters of chili

Significant effects of *M. javanica* and *R. solanacearum* and their interaction were observed in case of shoot and root lengths and weights of chili. The mean lengths and weights of shoot and root varied significantly as a result of both the pathogens applied singly and in combinations. The reductions in these parameters were significantly higher

when both the pathogens were applied simultaneously as compared to their sole applications. Similarly, at higher inoculum densities, the reductions were the maximum in all these parameters as shown in Table I.

Table I. Effect of *Ralstonia solanacearum* and *Meloidogyne javanica* on percent reduction in shoot length, root length, shoot weight and root weight and number of galls and number of egg masses on chilli roots. The table also shows wilt free days of chilli and incidence of bacterial wilt after 42 days.

| Meloidogyne javanica (J2s Kg ⁻¹) | Ralstonia solanacearum (cfu ml ⁻¹) | | | |
|--|--|------------------|-------------------|-------------------|
| | 0 | ×10 ⁵ | 1×10 ⁶ | 1×10 ⁷ |
| Shoot length | | | | |
| 0 | 0.00 | 5.56 | 14.81 | 18.15 |
| 1000 | 11.85 | 17.78 | 23.33 | 25.93 |
| 2000 | 23.33 | 25.56 | 29.26 | 30.74 |
| Root length | | | | |
| 0 | 0.00 | 7.91 | 11.8 | 19.24 |
| 1000 | 8.33 | 12.84 | 13.7 | 20.6 |
| 2000 | 11.17 | 21.24 | 23.4 | 26.17 |
| Shoot weight | | | | |
| 0 | 0.00 | 6.22 | 10.62 | 12.36 |
| 1000 | 7.48 | 11.34 | 13.83 | 15.98 |
| 2000 | 11.48 | 15.14 | 19.41 | 20.68 |
| Root weight | | | | |
| 0 | 0.00 | 6.78 | 9.56 | 16.38 |
| 1000 | 7.21 | 10.62 | 11.48 | 18.16 |
| 2000 | 9.35 | 13.83 | 16.36 | 21.24 |
| Number of galls on chilli roots | | | | |
| 0 | 0.00 | 0.00 | 0.00 | 0.00 |
| 1000 | 57 | 38 | 36 | 31 |
| 2000 | 72 | 47 | 42 | 37 |
| Number of egg masses on chilli roots | | | | |
| 0 | 0.00 | 0.00 | 0.00 | 0.00 |
| 1000 | 26 | 20 | 17 | 16 |
| 2000 | 34 | 26 | 20 | 17 |
| Wilt free days of chilli | | | | |
| 0 | 42 | 21 | 18 | 13 |
| 1000 | 42 | 19 | 15 | 11 |
| 2000 | 42 | 14 | 12 | 8 |
| Incidence of bacterial wilt after 42 days | | | | |
| 0 | 0.00 | 16.67 | 16.67 | 33.33 |
| 1000 | 0.00 | 16.67 | 33.33 | 50.00 |
| 2000 | 0.00 | 33.33 | 50.00 | 66.67 |

Effect on number of galls and egg masses

Significant variations were observed in number of galls and egg masses per root system in response to both the pathogens applied individually and in combinations. Maximum numbers of galls and egg masses were observed where nematode was applied alone. Significant reductions in galls and egg masses were recorded where nematode and the bacterium were applied in combinations. At higher densities of both the pathogens, the reductions in these parameters were the maximum as shown in Table I.

Effect on incidence of bacterial wilt

The first wilt appearance was observed after 8 days in the treatment where both the pathogens were applied at their highest densities. The treatments where bacterium was applied individually at different densities, wilt took maximum days to appear as compared to the treatments where bacterium and nematodes were applied in combinations as shown in Table I. Similarly, the incidence of bacterial wilt was lower where the nematode was absent. Minimum wilt incidence was recorded at the lowest density of *R. solanacearum*. The wilt incidence increased significantly when nematode was inoculated along with the bacterium and maximum incidence was recorded where both the pathogens were applied at their highest densities as shown in Table I.

DISCUSSION

The present study showed effects of *Ralstonia solanacearum* and *Meloidogyne javanica* individually and in combination. The combined effects of both the pathogens on chili were more pronounced as compared to the effects caused by single application of the pathogens. Similar results of increased bacterial wilt severity in the presence of *Meloidogyne* spp. were reported by many workers (Libman *et al.*, 1964; Jatala *et al.*, 1975; Sitaramaiah and Sinha, 1984). The reductions in shoot length and weight at pathogenic or above pathogenic density of *M. javanica* is due to the impaired carbohydrate partitioning to the roots (Carneiro *et al.*, 1999), mobilization and accumulation of photosynthesis products from shoots to roots during reproductive phase of adult females (Karssen and Moens, 2006). The findings confirmed the observations made by Sitaramaiah and Sinha (1984) who reported increase in reduction in plant heights in combined treatments of nematode and the bacterium in Brinjal.

Significant reductions in plant height, fresh shoot and root weights were observed in all the treatments in comparison to control. 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. javanica* played a significant role as a predisposing factor and thus increasing the incidence of wilt. Plants which received *M. javanica* and *R. solanacearum* separately as well as in three different combinations, recorded significantly lower plant height, fresh and dry shoot weight and fresh and dry root weight compared to healthy plants.

The bacterial wilt incidence was the maximum in plants inoculated simultaneously with nematode and the bacterium (Table I). Treatments with bacterium alone showed a decrease in wilt incidence. Similar results were reported by Routaray *et al.* (1986) who found that inoculation of *M. incognita* seven days prior to *P. solanacearum* at planting caused maximum wilt followed by simultaneous inoculations of both the pathogens 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. Similar results have also been reported by Haider *et al.* (1989) who reported that simultaneous inoculation of both the pathogens produced maximum wilt. Minimum wilt was noticed with bacterium alone. Further, Swain *et al.* (1987) 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, number of egg masses and root-knot index were the maximum for nematode treatment alone but it was significantly less in other treatments. Perhaps prior establishment of bacterial colonies in the rhizosphere was not conducive for nematode multiplication. Pitcher (1963) suggested that the bacterium modifies extensively host tissue which does not favor nematode multiplication. This could be the reason for decreased nematode multiplication in the present study. 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 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).

CONCLUSION

It is concluded from the present study that *Meloidogyne javanica* increased severity of bacterial wilt

caused by *Ralstonia solanacearum* at all the inoculum densities. The presence of *M. javanica* in bacterial inoculated plants accelerated the development of wilt symptoms. The simultaneous inoculation of both the pathogens also resulted in increase in reductions in growth parameters of chili, therefore, stringent control measures are recommended for the management of disease complex.

Statement of conflict of interest

The authors declare there is no conflict of interest.

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