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EFFECT OF SOLE AND COMBINED INOCULATION OF *RALSTONIA SOLANACEARUM* AND *MELOIDOGYNE JAVANICA* ON TOMATO

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ABSTRACT
The effects of *Meloidogyne javanica* alone and in different combination with *Ralstonia solanacearum* on tomato were investigated. The highest shoot length reduction (32.1%) was at the highest density of both the parasites over control. The highest initial population density of *M. javanica* was solely responsible for the highest root length reduction (33.25%) and shoot dry weight reduction (67.44%). The highest density of *R. solanacearum* is solely responsible for root fresh weight reduction (22.28-30.85%) irrespective of *M. javanica* densities. Higher density of *M. javanica* produced higher root fresh weight (1.69 - 1.96 g) in the absence of *R. solanacearum* over control (1.75 g). The gall intensity was dose dependent in absence of *R. solanacearum*. However, in the presence of *R. solanacearum* at its higher density, *M. javanica* produced lowest number of galls (20.9-34.7). The synergistic effects on percent wilt incidences (52 - 95%) were more prominent at lower levels of *R. solanacearum* population with pathogenic levels of *M. javanica*. Conclusively, the role of *M. javanica* is synergistic only if the density of *R. solanacearum* is lower than 10^5 cfug^{-1} soil, however, higher *R. solanacearum* population (10^7-10^9 cfug^{-1} soil) drastically inhibits the activity of root-knot nematode in the rhizosphere.

INTRODUCTION

Ralstonia solanacearum (Smith, 1896) Yabuuchi *et al.*, 1996 (hereinafter *R. s.*) one of the most destructive soil borne vascular pathogens of Solanaceous vegetables and several other crops grown in the tropical, subtropical as well as temperate regions of the world. In the soil ecosystem *R. s.* coexists with a large number of microorganisms, some favour the pathogen for their own interest, some inhibit them during the competition for space, nutrients and air. The involvement of nematodes in bacterial invasion is usually thought to be caused by wounds on the roots (Hayward, 1991). The concomitant infection by plant parasitic nematodes particularly sedentary endo-parasitic root-knot nematode (hereinafter RKN) and *R. s.* was long been reported to increase the severity of bacterial wilt (Napiere and Quimio, 1980; Cadet *et al.*, 1989; Deberdt, 1999; Hussain and Bora, 2009, Singh and Siddiqui, 2012, Siddiqui *et al.*, 2013). The combined pathogenic effects of *R. s.* and RKN were greater than the independent effects of either (Sitaramaiah and Sinha, 1984; Ateka *et al.*, 2001; Hussain and Bora, 2009). Chen (1984) reported changes the physiology of the plants due to nematode infestation predisposed tobacco plants to bacterial wilt. It was also suggested that RKN infestation greatly reduce the genetic resistance to bacterial wilt (Deberdt, 1999).

Hussain and Bora (2009) investigated the effect of different initial population densities of *Meloidogyne incognita* and *R. s.* on bacterial wilt of brinjal and found that the increased wilt incidence at higher inoculum level of the nematode and bacteria and low wilt incidence at lower inoculum levels of nematode with bacteria. In potato inoculated with *M. incognita* 10 days prior to *R. s.* inoculation showed higher bacterial wilt disease rating than those inoculated with both pathogens simultaneously, however, plants infected with either pathogen alone or together significantly reduced both root and shoot fresh weight (Bekhiet *et al.*, 2010). The investigation carried out by Singh and Siddiqui (2012) to study the effect of the order of inoculation of *Meloidogyne javanica* (hereinafter, *M. j.*) and *R. s.* on bacterial wilt and tomato growth revealed that the simultaneous inoculation or inoculation of *M. j.* 15 days prior to *R. s.* caused the greatest reduction in plant growth and highest wilt indices. More over, it was also noticed that the prior or simultaneous inoculation of *R. s.* with *M. j.* adversely affected the multiplication of *M. j.* in the soil and gall formation (Singh and Siddiqui, 2012, Siddiqui *et al.*, 2013).

All the preceding works have the notion towards additive effect of RKN on bacterial wilt development. These works considered only pre-determined specific combination of initial inoculum of both or either pathogen.

In the present investigation, the hypothesis is every possible combinations of initial inoculum densities, starting from 'below pathogenic', 'pathogenic' and 'above pathogenic' level, of either parasites have different effect on tomato growth, nematode multiplication and gall/egmass formation and bacterial wilt severity.

The present investigation was designed with the objective to determine how the different combination of initial population density of either or both the parasites at their 'below pathogenic', 'pathogenic' and 'above pathogenic' level affect the disease development and tomato growth.

MATERIALS AND METHODS

Sandy loam soil collected from Kalyani, Nadia, West Bengal

was added to 60 × 45 cm autoclavable plastic packet. The soil was moistened before transferring them to autoclave for sterilization at 121°C and 15 lbinch^{-2} pressure for 20 minutes. The sterilization process was repeated for three consecutive days. The sterilized soils were then allowed to cool down to room temperature and one kilogram of such soil was filled into each 15 cm diameter earthen pots (Singh and Siddiqui, 2012). Seeds of tomato, cv. Pusa Ruby were obtained from AICRP on Vegetable Crops, Kalyani Center and surface sterilized in 0.1% sodium hypochlorite for two minutes and washed three times with distilled water. Seeds were sown in seedling trays with sterilized soil. Twenty days old seedlings were transplanted at four seedling per pot and 20 pots per replication. Pots containing seedlings were placed in a poly-house and provided with 100 ml of water daily (Singh and Siddiqui, 2012). *R. s.* infected eggplants were collected and the pathogens were isolated. Infected plant parts were cut into small pieces, surface sterized with 0.1% $HgCl_2$ followed by three wash in sterile water and dipped in sterile water containing culture tubes to allow oozing. All operations were carried within Laminar Airflow chamber. After 15-20 minutes, the ooze in sterile water were streaked on *R. s.* semiselective SMSA (Englebrecht, 1994) with 0.005% 1,3,5 triphenyl tetrazolium chloride (TZC) following quadric streak method and/or dilution pore plate methods. Inoculated petri-plates were then allowed to incubate at 30 ± 1°C. *R. s.* produces fluidal colony with pink center and whitish periphery after 48 hours of incubation. Pure culture was isolated from such single colony on SMSA medium without TZC. The pathogen was identified through PCR with the primer pair 759F/760R and protocol described by Opina *et al.* (1997) followed by 2% agarose gel electrophoresis. The race of the pathogen was determined through *Capsicum* leaf infiltration method (Lozano and Sequeira, 1970) and Biovar of the isolate was determined based on the basis of utilization (oxidation and acid production) of disaccharide sugar and hexose sugar alcohols (Hayward, 1964). Race 1 (biovar III) isolate of *R. s.* was grown on SMSA (without TZC) slants for 48 hrs at 30°C. Sterile distilled water were flooded over the growth of bacterium allow to stand for 30 to 60 minutes so that, motile cells of the bacteria came in to the water. The pathogenicity of the isolate was confirmed by *in vitro* inoculation of tomato seedling (cv. Pusa Ruby) under laboratory condition. Bacterial suspension of 0.3 OD at 600 nm (equivalent to 1×10^8 cfu.ml^{-1}) was used for inoculation at 50 ml for each kilogram of soil (Hussain and Bora, 2009). RKN were collected from naturally infected brinjal plants and identified as *Meloidogyne javanica* (Treub) Chitwood by means of perineal pattern of adult female (Chitwood, 1949 and Taylor *et al.*, 1955) and prepared for inoculation following the method described by (Dasgupta, 1988). Roots with young galls were separated, washed them gently with tap water and were cut them into 2 to 5 cm length. The root pieces were placed in 0.5% (a.i.) sodium hypochlorite (NaOCl) solution at 20 g roots for each 200 ml of 0.5% NaOCl solution and shake or stir with motorized stirrer for 4 minutes. The NaOCl-egg suspension and roots were then placed over a 425 μm (40 mesh) sieve in to eight litter bucket and rinsed to collect freed eggs. Volume was made up to six litters. The egg suspension were then pore on to 26 μm (500 mesh) sieve and immediately rinse the eggs on 26 μm sieve with stream of cold tap water to remove residual NaOCl. Eggs were rinsed off-sieve and added water to desired concentration. Egg suspension was then placed on wire gauge covered with two layer of facial tissue paper and covered the eggs with sufficient water in a closed chamber. The chamber was constituted using a pair of Petri plates and the lower lid was filled with water upon which the wire gauge with nematode suspension was placed so that the lowers surface of the gauge was touching the upper level of water (Schindler, 1961 cit. Dasgupta, 1988) to facilitate the emergence of second stage infective juvenile (J2) and kept for 24 h. The emerged J2 were then counted in each millilitre of suspension and required concentration was obtained through dilution (Hussain and Bora, 2009). Inoculation was done immediately after collection of J2. The experiment was arranged in a completely randomized two factor factorial design, *M. j.* and *R. s.* inoculum were the factors with four (500 J2Kg^{-1} soil; 1000J2Kg^{-1} soil; 1500 J2Kg^{-1} soil and control) and five (3.3×10^{-4} cfu g^{-1} soil; 2.5×10^{-5} cfu g^{-1} soil; 1.7×10^{-7} cfu g^{-1} soil; 3.7×10^{-9} cfu g^{-1} soil and control) levels of initial population densities (IPD), respectively. The experiment was replicated thrice. Inoculations were done by surface drenching with the required concentration of *M. j.* J2 and *R. s.* suspension prepared in sterile distilled water, seven days after transplanting of tomato seedlings. The experiment was monitored regularly and wilt symptoms were recorded from time to time. Plant showing wilt symptoms were given the score of '100' Percent Wilt Incidence (PWI) and '0' PWI for plants not showing any wilt symptoms (Hussain and Bora, 2009). After 50 days of transplanting, the experiment was terminated and observations were recorded (Hussain and Bora, 2009). The individual plants were uprooted from pots carefully and washed gently in running tap water to remove adhering soil particles from the roots and care was taken so as to get the root system intact to avoid root damage. Excess water were blotted out and records were taken on root and shoot length, fresh and dry weight of shoots and roots, number of galls and number of egg masses per root system (Hussain and Bora, 2009). The shoot length, root length, and fresh weights were determined immediately after uprooting. The number of galls and eggmasses were counted under a dissecting microscope with 2.5× magnification. Following all quantitative measurements of fresh plant, the plants were cut in to shoots and roots and each portion were further cut into small pieces and wrapped separately in to brown paper packet with heat tolerant labels. The packets containing root and shoot pieces were then kept in to the hot air oven at 60 °C for consecutive three days until constant weights were obtained. Immediately after removal of the packets from hot air oven, dry weights were taken using electronic weighing scale.

RESULTS

Results indicated, there were no significant interaction effect on shoot length among different IPDs of either or both the parasites. The sole effects of *R. s.* differed significantly irrespective of different IPD of *M. j.* ($IPD_{M.\ j.}$) and *vice versa* (Table 1). The shoot length was the lowest (18.67 cm) when IPD of either parasite was at their highest level. The sole effect *R. s.* at $IPD_{R.\ s.}$ 2.5×10^5 cfug^{-1} soil or less, produced the highest shoot length (24.27 cm) and was at par with the control (23.75

cm) where both the parasites were absent. The shoot length was affected only by the higher population density of *R. s.* (10^7-10^9 cfug^{-1} soil), while the effects of $IPD_{M.\ j.}$ were dose dependent.

The average root length was not significantly varied with different IPDs of *R. s.*. However, the sole effect of different IPDs of *M. j.* varied significantly (Table 2). There was no significant variation in the interaction effect root length. The sole effect of $IPD_{M.\ j.}$ Control and 500J2Kg^{-1} produced the highest root length. The sole effect of $IPD_{M.\ j.}$ 1500 J2 Kg^{-1} soil was the most detrimental IPD and produced the lowest root length.

Shoot fresh weights did not vary significantly with different levels of IPD of *R. s.* and *M. j.* and there was no significant interaction effect on shoot fresh weight (data not shown). However, shoot dry weights varied significantly among all the predominant sole and interaction effect of $IPD_{R.\ s.}$ and $IPD_{M.\ j.}$ (Table 3). The maximum dry weight was observed in the pots received $IPD_{M.\ j.}$ 500J2Kg^{-1} soil and $IPD_{R.\ s.}$ 2.5×10^5 cfug^{-1} soil. However, the poorest interaction effect was between $IPD_{M.\ j.}$ 1500J2Kg^{-1} soil and $IPD_{R.\ s.}$ Control. The sole effect of $IPD_{M.\ j.}$ was at its lowest in 1500 J2Kg^{-1} soil and the sole effect of $IPD_{R.\ s.}$ was at its highest in 2.5×10^5 cfug^{-1}. $IPD_{M.\ j.}$ 1000 J2Kg^{-1} soil helps to increase shoot dry weight and both higher and lower initial densities of *M. j.* were effectively able to reduce the shoot dry weight. On the other hand the critical initial density of *R. s.* was 1.7×10^7 cfug^{-1} soil for dry weight reduction.

The minimum percent weight loss upon drying was 75.4% [10.8% below the average percent weight loss (83.40%)] in the case of $IPD_{R.\ s.}$ 3.7×10^9 cfug^{-1} soil and $IPD_{M.\ j.}$ 1000J2Kg^{-1} soil on drying while the highest weight loss (91.9%) was obtained in $IPD_{R.\ s.}$ Control and $IPD_{M.\ j.}$ 1500 J2Kg^{-1} soil, which is 9% above the average percent weight loss (Figure 1 and 2).

The average fresh weight of roots differed significantly in response to both the pathogens together and also with the sole effect of different $IPD_{M.\ j.}$ and $IPD_{R.\ s.}$. The sole effects of $IPD_{R.\ s.}$ were at their lowest at the highest IPD of the pathogen but the sole effect of $IPD_{M.\ j.}$ were at their lowest at the lowest IPD ($IPD_{M.\ j.}$ 500 J2Kg^{-1} soil) and was statistically at par with the root fresh weight in absence of both pathogen. The $IPD_{M.\ j.}$ at pathogenic or above pathogenic levels (1000 J2 Kg^{-1} soil)

Table 1: Effect of different IPDs of of *M. j.* and *R. s.* on shoot length (cm) of tomato

	$IPD_{M.\ j.}$ 500	$IPD_{M.\ j.}$ 1000	$IPD_{M.\ j.}$ 1500	$IPD_{M.\ j.}$ Control	Mean
$IPD_{R.\ s.3.3\times10^4}$	24.83	24.33	21.40	26.33	24.22^{p}
$IPD_{R.\ s.2.5\times10^5}$	25.67	23.80	22.10	25.50	24.27^{p}
$IPD_{R\ S1.7\times10^7}$	21.27	20.67	19.10	22.17	20.80^{q}
$IPD_{R.\ s.\ 3.7\times10^9}$	21.00	20.00	18.67	23.50	20.79^{q}
$IPD_{R.\ s.}$Control	25.20	22.20	20.10	27.50	23.75^{p}
Mean	23.59^{b}	22.20^{c}	20.27^{d}	25.00^{a}	
Source	SE (d)	CD at 5%			
R. s.	0.38	1.09			
M. j.	0.34	0.98			
*R. s.***M. j.*	0.77	NS			

a-d and p-t sets of letters have been used to differentiate the effect sizes on the basis of DMRT in rows and columns, respectively. Data bearing same letter are not significantly different at 5% level of significance

Table 2: Effect of different IPDs of *M. j.* and *R. s.* on root length (cm) of tomato

	$IPD_{M.\ j.}$ 500	$IPD_{M.\ j.}$ 1000	$IPD_{M.\ j.}$ 1500	$IPD_{M.\ j.}$ Control	Mean
$IPD_{R.\ s.3.3\times10^4}$	11.85	11.12	10.20	12.47	11.41
$IPD_{R.\ s.2.5\times10^5}$	13.17	12.40	9.33	13.67	12.14
$IPD_{R\ S1.7\times10^7}$	10.67	10.20	9.33	12.20	10.60
$IPD_{R.\ s.\ 3.7\times10^9}$	12.33	11.47	8.67	13.10	11.39
$IPD_{R.\ s.}$Control	11.83	10.60	8.25	12.36	10.76
Mean	11.97^{ab}	11.16^{b}	9.16^{c}	12.76^{a}	
Source	SE(m)	CD at 5%			
R. s.	0.38	NS			
M. j.	0.34	0.98			
*R. s.***M. j.*	0.77	NS			

Data bearing same letters are not significantly different at 5% level of significance

Table 3: Effect of different IPDs of *M. j.* and *R. s.* on shoot dry weight (g) of tomato

	$IPD_{M.\ j.}$ 500	$IPD_{M.\ j.}$ 1000	$IPD_{M.\ j.}$ 1500	$IPD_{M.\ j.}$ Control	Mean
$IPD_{R.\ s.3.3\times10^4}$	0.75^{bq}	0.92^{aq}	0.97^{ap}	0.78^{br}	0.85^{q}
$IPD_{R.\ s.2.5\times10^5}$	1.16^{ap}	0.75^{cr}	0.95^{bp}	0.88^{bpq}	0.94^{p}
$IPD_{R\ S1.7\times10^7}$	0.61^{br}	0.67^{bs}	0.53^{cr}	0.77^{ar}	0.64^{r}
$IPD_{R.\ s.\ 3.7\times10^9}$	0.64^{dr}	1.05^{ap}	0.79^{cq}	0.94^{bp}	0.86^{q}
$IPD_{R.\ s.}$Control	0.43^{b}	0.42^{bt}	0.28^{cs}	0.86^{aq}	0.50^{s}
Mean	0.72^{c}	0.76^{b}	0.70^{c}	0.85^{a}	
Source	SE(m)	CD at 5%			
R. s.	0.013	0.04			
M. j.	0.012	0.03			
*R. s.***M. j.*	0.026	0.07			

a-d and p-t sets of letters have been used to differentiate the effect sizes on the basis of DMRT in rows and columns, respectively. Data bearing same letters are not significantly different at 5% level of significance

produce significantly different root fresh weight and was at their highest in highest $IPD_{M.\ j.}$. Thus, notwithstanding minor variation, higher initial population of *R. s.* is solely responsible for fresh weight reduction irrespective of different *M. j.* population levels whereas, the higher initial inoculum levels of *M. j.*, interestingly, increased root fresh weight (Table 4).

The root dry weight did not vary significantly with different levels of IPD of *R. s.* and *M. j.* and there was no interaction effect on root dry weight (data not shown).

The number of galls per root system in response to the effect of all the predominant factors and their interactions varied significantly (Table 5). The effect size of sole $IPD_{R.\ s.}$ was at its lowest at 3.7×10^9 cfug^{-1} soil, while the effect size of sole $IPD_{M.\ j.}$ was at its highest in $IPD_{M.\ j.}$ 1500 J2Kg^{-1} soil and was statistically at par with $IPD_{M.\ j.}$ 1000J2Kg^{-1} soil when *R. s.* was absent. However, presence of *R. s.* in the soil, the galling intensity did not vary significantly with different $IPD_{M.\ j.}$ (Table 5). Interestingly, the galling intensity reduced at the lowest level (20.9 galls per root system) with the highest IPD of both the pathogens (Table 5). Similarly, the eggmass production did not vary significantly with the sole effect of different IPD level of *M. j.* (Table 6). The interaction effects of $IPD_{M.\ j.}$ 1000 & 1500 J2Kg^{-1} soil and $IPD_{R.\ s.}$ 1.7×10^7 & 3.7×10^9 cfug^{-1} soil significantly reduced the eggmass production in respect to the pots received only *M. j.* at 1000 & 1500 J2Kg^{-1} soil.

The percent wilt incidence (PWI) was found higher at $IPD_{R.\ s.}$ 3.7×10^9 cfug^{-1} soil irrespective of different levels of $IPD_{M.\ j.}$ (Table 7). Significantly lower PWI was observed when *M. j.* was absent. This difference in the outcome indicates the aggregative effect of *M. j.* on disease development. The effect size was increased with increasing level of both the parasites. The synergistic effects were more prominent at lower levels of *R. s.* population with pathogenic levels of *M. j.* (1000 J2K^{-1} soil).

First wilt incidence occurred at 8-9 days after inoculation at all the inoculum levels of *M. j.* and were statistically at par.

Table 4: Effect of different IPDs of *M. j.* and *R. s.* on root fresh weight (g)

	$IPD_{M.\ j.}$ 500	$IPD_{M.\ j.}$ 1000	$IPD_{M.\ j.}$ 1500	$IPD_{M.\ j.}$ Control	Mean
$IPD_{R.\ s.3.3\times10^4}$	1.53^{bq}	1.62^{aq}	1.49^{bq}	1.50^{br}	1.54^{r}
$IPD_{R.\ s.2.5\times10^5}$	1.57^{bq}	1.66^{aq}	1.40^{cr}	1.65^{aq}	1.57^{q}
$IPD_{R\ S1.7\times10^7}$	1.42^{abr}	1.37^{br}	1.26^{cs}	1.47^{ar}	1.38^{s}
$IPD_{R.\ s.\ 3.7\times10^9}$	1.35^{abr}	1.28^{bcs}	1.21^{cs}	1.36^{as}	1.30^{t}
$IPD_{R.\ s.}$Control	1.69^{cp}	1.82^{bp}	1.96^{ap}	1.75^{bcp}	1.81^{p}
Mean	1.51^{b}	1.55^{a}	1.46^{c}	1.55^{a}	
Source	SE(m)	CD at 5%			
R. s.	0.013	0.04			
M. j.	0.012	0.03			
*R. s.*M. j.*	0.026	0.07			

a-d and p-t sets of letterss has been used to differentiate the effect sizes on the basis of DMRT in rows and columns, respectively. Data bearing same letters are not significantly different at 5% level of significance

Table 5: Effect of different IPDs of *M. j.* and *R. s.* on number of gall per root system of tomato

	$IPD_{M.\ j.}$ 500	$IPD_{M.\ j.}$ 1000	$IPD_{M.\ j.}$ 1500	$IPD_{M.\ j.}$ Control	Mean
$IPD_{R.\ s.3.3\times10^4}$	$52.67(7.27)^{apq}$	$62.40(7.91)^{aq}$	$48.9(7.01)^{aq}$	$0.00(0.71)^{b}$	$32.30(5.72)^{q}$
$IPD_{R.\ s.2.5\times10^5}$	$56.67(7.55)^{abpq}$	$65.60(8.12)^{aq}$	$40.3(6.37)^{bqr}$	$0.00(0.71)^{c}$	$31.90(5.69)^{q}$
$IPD_{R.\ s.1.7\times10^7}$	$42.40(6.54)^{aq}$	$37.3(6.12)^{ar}$	$26.20(5.16)^{abr}$	$0.00(0.71)^{c}$	$21.00(4.63)^{r}$
$IPD_{R.\ s.\ 3.7\times10^9}$	$34.70(5.93)^{aq}$	$28.3(5.92)^{ar}$	$20.9(4.50)^{as}$	$0.00(0.71)^{b}$	$16.40(4.11)^{s}$
$IPD_{R.\ s.}$Control	$69.40(8.34)^{bp}$	$112.70(10.63)^{ap}$	$135.6(11.65)^{ap}$	$0.00(0.71)^{c}$	$60.9(7.83)^{p}$
Mean	$50.30(7.13)^{b}$	$57.50(7.61)^{a}$	$47.70(6.94)^{b}$	$0.00(0.71)^{c}$	
Source	SE (m)	CD at 5%			
R. s.	0.153	0.44			
M. j.	0.137	0.39			
*R. s.*M. j.*	0.306	0.87			

Figures in the parentheses are square root transformed ($\sqrt{Y+0.5}$), a-d and p-t sets of letters has been used to differentiate the effect sizes on the basis of DMRT in rows and columns, respectively. Data bearing same letters are not significantly different at 5% level of significance

Table 6: Effect of different IPDs of *M. j.* and *R. s.* on number of egg mass per root system

	$IPD_{M.\ j.}$ 500	$IPD_{M.\ j.}$ 1000	$IPD_{M.\ j.}$ 1500	$IPD_{M.\ j.}$ Control	Mean
$IPD_{R.\ s.3.3\times10^4}$	$44.30(6.67)^{ap}$	$50.28(7.13)^{apqr}$	$37.15(6.14)^{apq}$	$0.00(0.71)^{b}$	$26.18(5.17)^{q}$
$IPD_{R.\ s.2.5\times10^5}$	$44.55(6.71)^{ap}$	$79.89(8.63)^{apq}$	$34.33(5.90)^{abq}$	$0.00(0.71)^{c}$	$29.61(5.49)^{q}$
$IPD_{R.\ s.1.7\times10^7}$	$33.62(5.84)^{ap}$	$28.04(5.34)^{apr}$	$22.83(4.83)^{aq}$	$0.00(0.71)^{b}$	$16.97(4.18)^{r}$
$IPD_{R.\ s.\ 3.7\times10^9}$	$23.80(4.93)^{apq}$	$18.29(4.34)^{ar}$	$18.16(4.33)^{aq}$	$0.00(0.71)^{b}$	$12.29(3.58)^{s}$
$IPD_{R.\ s.}$Control	$66.81(8.20)^{ap}$	$75.14(8.70)^{ap}$	$87.84(9.40)^{ap}$	$0.00(0.71)^{b}$	$45.09(6.75)^{p}$
Mean	$41.44(6.48)^{a}$	$46.08(6.83)^{a}$	$36.95(6.12)^{a}$	$0.00(0.71)^{b}$	
Source	SE (m)	CD at 5%			
R. s.	0.344	0.98			
M. j.	0.307	0.88			
*R. s.*M. j.*	0.687	1.96			

Figures in the parentheses are square root transformed ($\sqrt{Y+0.5}$), a-d and p-t sets of letters has been used to differentiate the effect sizes on the basis of DMRT in rows and columns, respectively. Data bearing same letters are not significantly different at 5% level of significance

Table 7: Effect of different IPDs of *M. j.* and *R. s.* on PWI of tomato

	$IPD_{M.\ j.}$ 500	$IPD_{M.\ j.}$ 1000	$IPD_{M.\ j.}$ 1500	$IPD_{M.\ j.}$ Control	Mean
$IPD_{R.\ s.3.3\times10^{4}}$	28.0(31.90)^{cs}	52.0(46.10)^{as}	44.0(41.67)^{bs}	27.0(31.30)^{cs}	38.0(37.73)^{s}
$IPD_{R.\ s.2.5\times10^{5}}$	48.0(43.90)^{cr}	67.0(54.90)^{ar}	61.0(51.40)^{br}	39.0(38.6)^{dr}	54.0(47.19)^{r}
$IPD_{R\ S1.7\times10^{7}}$	72.0(58.10)^{cq}	77.0(61.30)^{bq}	99.0 (85.2)^{aq}	52.0(46.10)^{dq}	75.0(62.67)^{q}
$IPD_{R.\ s.\ 3.7\times10^{9}}$	92.0(73.60)^{cp}	95.0(77.10)^{bp}	100.0(90.00)^{ap}	58.0(49.60)^{dp}	86.0(72.01)^{p}
$IPD_{R.\ s.}$Control	0.0(0.37)^{t}	0.0(0.37)^{t}	0.0(0.37)^{t}	0.0(0.37)^{t}	0.0(0.37)^{t}
Mean	48.0(41.57)^{c}	58.0(47.98)^{b}	61.0(53.23)^{a}	35.0(33.21)^{d}	
Source	SE (m)	CD at 5%			
R. s.	0.416	1.19			
M. j.	0.372	1.06			
*R. s.*M. j.*	0.833	2.38			

Figures in the parentheses are angular ($Sin^{-1}\sqrt{p}$) transformed, a-d and p-t sets of letters has been used to differentiate the effect sizes on the basis of DMRT in rows and columns respectively. Data bearing same letters are not significantly different at 5% levels of significance

Table 8: Effect of different IPDs of *M. j.* and *R. s.* on number of wilt free days of tomato

	$IPD_{M.\ j.}$ 500	$IPD_{M.\ j.}$ 1000	$IPD_{M.\ j.}$ 1500	$IPD_{M.\ j.}$ Control	Mean
$IPD_{R.\ s.3.3\times10^{4}}$	23.00^{aq}	17.00^{bcq}	15.00^{cq}	26.00^{aq}	20.25^{q}
$IPD_{R.\ s.2.5\times10^{5}}$	19.00^{ar}	15.00^{bcq}	12.00^{cqr}	21.00^{ar}	16.75^{r}
$IPD_{R\ S1.7\times10^{7}}$	13.00^{as}	10.00^{ars}	10.00^{ars}	12.00^{as}	11.25^{s}
$IPD_{R.\ s.\ 3.7\times10^{9}}$	9.00^{at}	8.00^{as}	8.00^{as}	11.00^{at}	9.00^{t}
$IPD_{R.\ s.}$Control	#50.00^{ap}	50.00^{ap}	50.00^{ap}	50.00^{ap}	50.00^{p}
Mean	22.80^{a}	20.00^{b}	18.00^{b}	24.00^{a}	
Source	SE(m)	CD at 5%			
R. s.	0.624	1.78			
M. j.	0.558	1.60			
*R. s.*M. j.*	1.248	3.57			

a-d and p-t sets of letters has been used to differentiate the effect sizes on the basis of DMRT in rows and columns respectively . Data bearing same letters are not significantly different at 5% levels of significance, # the experiment was terminated at 50 Days after inoculation

However, the longest time for symptom appearance was required at the lowest level of *R. s.* in the absence of *M. j.* (Table 8).

DISCUSSION

The effect responses of either parasite alone were more or less similar in terms of shoot length. However, presence of both parasite was aggravated the situation particularly when both were at their highest levels of density tested. These clearly explain the statistically insignificant variation in the interaction effect of either parasite. Thus, in the case of *M. j.*, the shoot length and shoot dry weight reduction at pathogenic or above pathogenic density level strongly influenced by the impaired carbohydrate partitioning to the roots (Carneiro *et al.*, 1999), mobilization and accumulation of photosynthesis products from shoots to roots during egg laying periods of adult female (Karssen and Moens, 2006), The present finding surely confirms the observation made by Sitaramaiah and Sinha (1984) where they stated the increased reduction in plant heights in combined treatments of *M. j.* and *R. s.* in Brinjal, but only at higher level of initial density. The present findings did not corroborate the results showed by Hussain and Bora (2009) that *M. incognita* in brinjal was solely responsible for shoot length reduction alone or in combination with *R. s.*. As it is found that results differed with RKN species (Maleita *et al.*, 2012), further investigation is of worth to elucidate the effect of both parasites at their specific and/or sub-specific levels.

The results of the present investigation showed that *M. j.* was solely responsible for root length reduction and *R. s.* have no effect in this parameter of tomato growth. Lower level of IPD of *M. j.* (500 J2Kg^{-1} soil) apparently have no effect on root lengths, presumably due to the intrinsic compensation responses of the plant. However, the intrinsic response of the plant compromised with progressive increase in initial population densities (two and three fold) (Table 2). The present findings corroborate with the findings of Maleita *et al.* (2012). This is most obviously due to the primary damage to the attacked roots or invasion of *M. j.* leading to the withdrawal of nutrients and impaired physiological aspects. The results clearly indicated that *M. j.* invasion at their pathogenic or above pathogenic density reduced root elongation that ultimately resulted in decreased uptake of water and nutrients (Karssen and Moens, 2006; Maleita *et al.*, 2012). The effects were worse with the reduction in root efficiency resulted in decrease in root shoot ratio. The present findings also corroborate with observations made by Trudgill (1991) that plant generates more roots to over come the limitations due to RKN damage, but only when the initial population density is below pathogenic level.

Neither pathogen was able to affect shoot fresh weight and root dry weight in comparison to the absence of either or both the pathogens together and contradicted Hussain and Bora (2009) who observed significant variations in these parameters. The prominent increase in root fresh weight in the absence of *R. s.* was probably due to gall formation and secondary root proliferation (Carneiro *et al.*, 1999). This is due to the fact that RKN infected tissues (galls) contain highly dense granular protoplasm (Trudgill, 1991). This finding is in confirmation with Hussain and Bora (2009) and Maleita *et al.* (2012).

A progressive increase in number of galls and egg masses with the increased inoculum level of the nematode from below-pathogenic level to pathogenic level was recorded by Hussain and Bora (2009). However, in the present experiment, it was

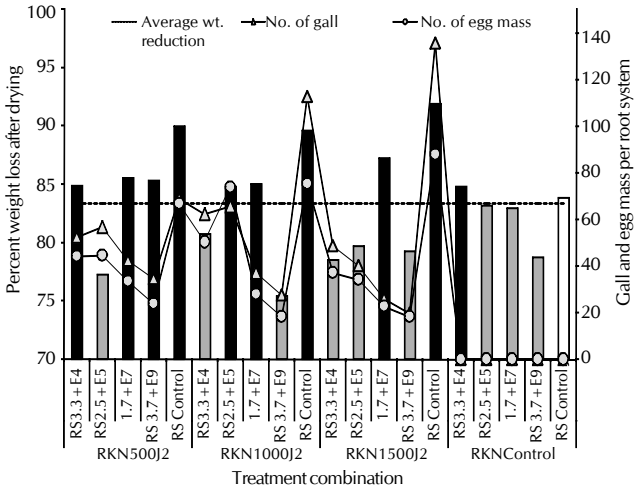

Figure 1: Relationship between percent shoot weight reduction during drying (dark gray fill = above average, light gray = below average, white = control), [+E4 = 10^4, +E5 = 10^5, + E7 = 10^7, +E9 = 10^9]; *R. s.* = *Ralstonia solanacearum* race 1, biovar III, RKN = Root Knot Nematode (*Meloidogyne javanica*)

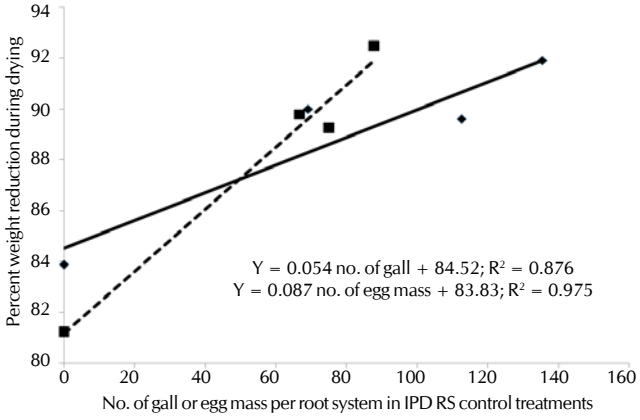

Figure 2 Relationship between percent shoot weight reduction during drying with number of galls per root system (●) and number of eggmass per root system (▬). [IPD = Initial Population Density, *R. s.* = *Ralstonia solanacearum*]

found that in absence of *R. s.*, number of gall per root system with pathogenic (1000J2Kg^{-1} soil) and above pathogenic (1500J2Kg^{-1} soil) are statistically at par. This is perhaps due to the intra-specific competition that limits the unfit candidate to produced gall. On the other hand, the results indicated that higher IPD of *R. s.* solely govern the eggmass production. Hussain and Bora (2009) also observed that the nematode alone produced more galls and egg masses compared when they are in association with bacteria. The present findings corroborate this observation made by Hussain and Bora (2009), Bhagawati *et al.* (1996) and Hazarika (2003) who reported significantly poor galls and egg masses in jute when *M. incognita* was associated with *R. solanacearum*, but it is in disagreement with the finding of Sellam *et al.* (1980) who reported that presence of the bacteria did not effect the root galling in tomato plants and at the same time established the responsibility of *R. s.* at higher density hinders the *M. j.* population by means of toxic secondary metabolite production in the rhizosphere, promoting competition for space and food and rendering root surface unfavourable for probing and subsequent feeding (Turner *et al.*, 2009).

The percent weight reduction during drying was not similar to each other (Figure 1). It was found that the percent weight reduction during drying equals the average weight reduction when both the parasites were absent. Otherwise, they are either below or above average line. It was also revealed from figure 1 that number of gall and eggmass per root system are directly proportional to higher percent weight reduction. The possible explanation is that the numbers of successful infection (indicated by gall formation along with eggmass production) by *M. j.* significantly affect the retention of absorbed water in the plant tissue presumably due to the encouragement of rate of assimilation over transpiration, thereby increasing tissue water content in its turn. This may be due to higher water use efficiency of nematode infested plants along with low transpiration due to abnormal stomatal closure as the intrinsic response of plants toward nematode infestation (Tahery, 2012). High IPD of *R. s.* inhibited *M. j.* infestation (Swain *et al.*, 1987; Hussain and Bora, 2009), prevented intrinsic response and encouraged normal gas exchange and transpiration by provoking stomata openings. Thus, tissue water retention was negligible. However, the effect of high initial population of *R. s.*, in soil or on the root surface, on the above ground part is still unclear. Nevertheless, the possibility of suppression of the pathogen associated molecular pattern (PAMP) trigerred immunity (PTI) with the type III effector(s) of virulent pathogen and so prevention of stomatal closure (Göhre and Robatzek, 2008), ultimately resulted in regular transpiration and low water retention within the plant system, could also not been ruled out. This indicated the responsibility of higher $IPD_{R.\ s.}$ in the reduction of galling intensity. The trend prevails irrespective of different $IPD_{M.\ j.}$ in absence of *R. s.*. This finding unequivocally confirms the previous findings that the higher population density of *R. s.* hinders the *M. j.* due to toxic secondary metabolites production in the soil, promotes competition for space, food and renders root surface unfavourable for probing and subsequent feeding (Sitaramaiah and Sinha, 1984; Swain *et al.*, 1987; Bhagawati *et al.*, 1996; Hazarika, 2003;Turner *et al.*, 2009; Hussain and Bora, 2009; Singh and Siddiqui, 2012).

The highest PWI was obtained when the *M. j.* was inoculated at above-pathogenic level (1500 J2Kg^{-1} soil) with all the levels of *R. s.* (Table 7), which is in agreement with Hussain and Bora (2009) who observed the same at higher than pathogenic level (2000 J2Kg^{-1} soil). However, none of the other associations of these two pathogens as well as bacterium alone could produce 100% wilt incidence even at the termination of the crop (50 days of inoculation). The increased wilt incidence at higher inoculum level of *M. j.* and *R. s.* could be attributed to increase puncturing of root by the nematode which facilitates increased establishment and multiplication of the bacterium in host (Hussain and Bora, 2009). Similarly, low wilt incidence at lower inocula levels of *M. j.* with *R. s.* may be due to decrease in root puncturing by the *M. j.* (Hussain and Bora, 2009). Similar findings were also reported by Napier and Quimo (1980) in tomato and Sitaramaiah and Sinha (1984) in brinjal.

The present findings confirm the intelligent speculations made by Singh and Siddiqui (2012) that interactions between these pathogens may have both direct and indirect effects on disease

severity. The direct effects include physical interactions of pathogens in the rhizosphere and occupancy of the same infection site in- and outside the root surface have had an antagonistic effect on *M. j*.. Indirect effects of interactions via plant response, such as breaking of disease resistance, and modification of host substrate had synergistic effects on disease severity. Plant parasitic nematodes cause physical damage that can allow secondary infection by other pathogens (Sitaramaiah and Pathak, 1993).

The effects of the interaction in terms of wilt free days clearly indicate that the aggregative effect of *M. j*. not only increase the wilt severity at higher population level but also hastens the symptom development. The difference between highest population level of *M. j*. and *M. j*. IPD Control again indicated that the aggravation is more prominent in lower population levels of *R. s*..

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