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Epidemiological studies on tomato bacterial wilt incited by *Ralstonia solanacearum*

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Abstract
Bacterial wilt of tomato caused by *Ralstonia solanacearum*, is responsible for causing severe losses in tomato crops around the world. It has been reported that *Ralstonia solanacearum* may conquer 450+ plant species from 54 different botanical families, with solanaceous crops being its most vulnerable hosts. In the present study, the effect of different temperature, moisture, pH and inoculums load of *R. solanacearum* to cause bacterial wilt disease under control conditions was undertaken. Epidemiological studies revealed rapid bacterial wilt development at higher temperature levels (30-35 °C). A significant decline in disease progression was noticed at temperature below 30 °C. The rapid bacterial wilt development was found at acidic (pH<6). Decrease in disease progression was seen at higher (pH >7). The moisture level of 90 percent was found ideal for the disease development. The incidence of the disease was greatly reduced under drier soil conditions i.e. 20 to 30 percent of its water holding capacity. The disease severity was found maximum at inoculums level of 10^8 and 10^{10}c.f.u/ml. Root injury favours the disease development.

Keywords: Tomato, *Ralstonia solanacearum*, temperature, moisture

Introduction
Tomato (*Lycopersicon esculentum* L.) is one of the most important vegetable crop in the world and rich source of antioxidants, vitamin C, β-carotene and lycopene. Among the major diseases of tomato, bacterial wilt caused by *R. solanacearum* is a lethal wilting disease. The pathogen exhibit wide host range with 450 host species in 54 botanical families in tropical and subtropical regions of the world (Allen *et al*. 2005; Singh *et al*. 2011) [1, 14]. It frequently results in yield losses of over 90% during the warm, rainy months (Dharmatti *et al*. 2009) [4]. Temperature, moisture, pH level, inoculums pressure, and other variables all have an impact on the incidence and severity of bacterial wilt. When ambient temperatures are high, plants that were resistant to *R. solanacearum* at a moderate temperature become more vulnerable (Hayward 1991; Prior *et al*. 1996) [6, 12]. The severity of the bacterial wilt disease in crops is influenced by the damage to the root system, which can be caused by insects, nematodes, or both. The presence of inoculums of the pathogen in the plant's rhizosphere affects the occurrence of wilt disease in tomato and other crops (Kishun & Chand 1990) [10]. The lack of an effective detection tool, particularly when the pathogen density is either very low or there are no signs of the disease, greatly impedes epidemiological investigations on the population densities and distribution of *R. solanacearum* in the plant system. A meager information is available on development of tomato bacterial wilt in regard of abiotic factors. Therefore, in order to understand the relationship between the pathogen, environment and host, this study aims to determine the impact of different temperature, pH, moisture, and inoculums potential *R. solanacearum* on the disease intensity, population and movement of pathogenic bacteria as well as other bacteria in tomato cultivars that are both moderately resistant and susceptible.

Material and Methods
Effect of different temperature regimes on disease development
In order to find out the optimum temperature for disease development, the established seedlings in pots (7.5 cm dia.) with and without root injury were inoculated by using drench method and incubated at different temperatures *viz*. 20, 25, 30, 35 and 40 °C in relative humidity cum temperature control cabinet in separate sets. Nine plants were taken per replication and the treatments were replicated three times.

The data on bacterial wilt incidence were recorded after 11 days of incubation as per scale given by Winstead and Kelmen (1952) [17] where 1 =no symptom; 2=one leaf wilted; 3 =two or three leaf wilted; 4 =four or more leaf wilted; 5=whole plant wilted. Percent disease index (PDI) was calculated as per the following formula given below:

$$\text{Percent disease index (PDI)} = \frac{\sum(\text{ni x vi})}{\text{V X N}} \text{ x } 100$$

Where,
ni = number of plants with respective disease rating
vi = disease rating
V = the highest disease rating
N = the number of plants observed

Effect of different pH levels on disease development

To study the effect of different pH levels on the bacterial wilt disease development, different pH levels viz. 4.0, 5.0, 6.0, 7.0 and 8.0 were maintained in pot filled in with sterilized soil using different concentration of hydrochloric acid or sodium hydroxide. The established seedlings in pots adjusted at different pH levels were inoculated by using drench method and incubated at 30±2 °C in relative humidity cum temperature control cabinet. The data on percent disease index (PDI) as per the scale given by Winstead and Kelmen (1952) [17].

Effect of different moisture levels on disease development

In order to study the effect of different soil moisture levels viz., 25, 50, 75 & 90 percent of WHC, the soil taken from field was oven dried at 105 °C and its water holding capacity (WHC) was determined by saturating the soil, by using formula as given below: Accordingly the soil moisture levels of 25, 50, 75 & 90 percent of WHC were maintained in pots filled in with sterilized soil in different sets. The established seedlings in respective pots adjusted at different moisture levels were inoculated by using drench method and incubated at 30±2 °C in relative humidity cum temperature control cabinet. The data on percent disease index (PDI) as per the scale given by Winstead and Kelmen (1952) [17].

$$\text{WHC} = \frac{\text{Gain in weight at saturation point}}{\text{Dry weight of the soil}} \text{ x } 100$$

Effect of different inoculums pressure on disease development

To study the effect of different inoculums pressure on the bacterial wilt disease development, different concentration of inoculums were prepared (10^2, 10^4, 10^6, $10^8$and 10^{10}) by using serial dilution method. The established seedlings in pots were inoculated with different inoculums concentration levels by using drench method and incubated at 30±2 °C in relative humidity cum temperature control cabinet. The data on percent disease index (PDI) as per the scale given by Winstead and Kelmen (1952) [17].

Result and Discussion

Effect of different temperature regimes on disease development

To study the effect of various temperatures regimes on bacterial wilt disease development in tomato, 21 days old seedlings were transplanted in pots and inoculated with bacterial suspension ($3.0x10^8$c.f.u/ml) by using drench method. Then these pots were kept at various temperature regimes viz. 20, 25, 30, 35 and 40 °C. Observations on percent disease index were recorded after 11 days after transplanting and percent disease index was calculated and presented in Table 1.

Table 1: Effect of different temperature regimes on bacterial wilt development in tomato

Temperature (°C)	Disease severity index (%)		Mean
	Injured roots	Uninjured roots	
20	20.00 (26.55)	20.00 (26.55)	20.00 (26.55)
25	26.66 (31.00)	24.00 (29.20)	25.33 (30.10)
30	90.66 (76.15)	80.00 (63.92)	85.33 (70.03)
35	96.00 (82.20)	89.33 (72.93)	92.66 (77.57)
40	0.00 (4.83)	0.00 (4.83)	0.00 (4.83)
Mean	46.66 (44.15)	42.66 (39.49)	

Values given in brackets are the arcsine transformed values
CD $_{0.05}$ Temperature (T) =5.99, Inoculation method (I) =3.79, T X I= 7.45

The perusal of data (Table 1) revealed a varied disease progression response of bacterial wilt at different temperature regimes studied. Irrespective of the method of inoculation, temperature level of 35 °C proved most favorable for bacterial wilt development followed by that of 30 °C. A significant decline in disease progression was noticed with further decline in temperature below 30 °C. Symptoms of bacterial wilt were not observed at higher temperature level of 40 °C. The study further revealed that irrespective of temperature levels, the bacterial wilt progression was higher in injured roots as compared to uninjured roots.

The results of present studies are in conformity with Bittner *et al*. (2016) [2] who also observed no symptoms of bacterial wilt at 10 and 15 °C, while recorded highest disease incidence at 30 and 35 °C. Champoiseau and Momol (2009) [3] reported that temperature between (29-35 °C) played a important role in pathogen growth as well as in disease development. Fajinmi and Fajinmi (2010) [5] clearly reported that bacterial wilt pathogen was most serious on tomato plants, when temperature ranged between 30 °C and 35 °C. Mondal *et al*. (2014) [11] also reported the maximum bacterial wilt disease intensity at temperature ranging between 30° and 35 °C.

Effect of different soil moisture levels on disease development

The study (table 2) revealed an increasing bacterial wilt progression with the increasing soil moisture level, being highest (97.60%) at 90% soil moisture level of water holding capacity and lowest (20.00%) at 25% soil moisture level. A significant rapid disease progression was noticed at soil moisture levels above 50%. Except at lower soil moisture level of 25%, the injured roots exhibited higher response to bacterial wilt development as compared to uninjured roots at all soil moisture levels studied.

Table 2: Effect of different soil moisture levels on bacterial wilt development in tomato

Moisture level (% WHC)	Disease severity index (%)		Mean
	Injured roots	Uninjured roots	
25	20.40(26.84)	20.00(26.55)	20.20(26.70)
50	40.80(39.68)	32.80(37.32)	36.80(38.50)
75	76.80(61.20)	68.60(57.78)	72.20(59.49)
90	97.60(82.87)	89.80(75.86)	93.70(79.37)
Mean	58.90(52.65)	52.80(49.38)	

Figures in the parentheses are arc sine transformed values
CD$_{0.05}$ Moisture level (M) = 3.25, Inoculation method (I) = 2.30, M

X I= 4.78

The results of present study are in conformity with the findings of Hingorani *et al*. (1956) who observed that rapid development of the disease was favored with increase in soil moisture content from 50 to 100 percent of its water holding capacity. Similar result also have been reported by Sabet and Baraket (1971) who found that moisture level of 90 percent was ideal for disease development in potatoes. Similarly, Islam and Toyota (2004) reported that survival and wilt incidence of the pathogen was greatly reduced under drier soil conditions i.e. 20 to 30 percent of its water holding capacity.

Effect of different pH levels on disease development

From the data (Table 3), it is evident that the different pH levels exhibited significantly varied response for bacterial wilt development. The pH level of 5.0 favored the bacterial wilt progression most closely followed by that of pH 6.0. The disease development being only moderate (41.80% to 46.80) at pH 7 and pH 4 while it was minimum (24.00%) at pH 8. Irrespective of different pH level studies, the higher disease progression was observed in injured roots compare to that of uninjured roots.

Table 3: Effect of different pH levels on disease development in tomato

pH level	Disease severity index(%)		Mean
	Injured roots	Uninjured roots	
4	45.60(42.45)	38.00(38.01)	41.80(40.23)
5	93.20(76.31)	84.80(67.18)	89.00(71.75)
6	90.80(72.92)	80.00(63.53)	85.40(68.23)
7	52.00(46.13)	41.60(37.07)	46.80(29.55)
8	26.80(31.18)	21.20(27.39)	24.00(27.53)
Mean	61.68(53.79)	53.12(46.64)	

$CD_{0.05}$ pH level (P) = 3.38, Inoculation method (I) = 2.14, P X I = 4.98

The results of the present study are in agreement with the findings of Kelmen (1953) who reported the occurrence of bacterial wilt more common in moderate acidic soil (pH 5-5.5). Similarly Prior *et al*, (1996) [12] also recorded higher bacterial wilt disease development in soil with acidic pH ranging between 5 to 5.5. Vincent *et al*. (1997) [16] also reported suppressed growth of all strains of *Ralstonia solanacearum* was suppressed at pH 3, 10, 11 and strongly reduced at pH 4 and 9.

Effect of different inoculums pressure on disease development

The result of the study (Table 4) revealed a increasing infection response with the increasing inoculums concentrations from 10^2c.f.u/ml to 10^{10}c.f.u/ml. The increasing inoculums levels from 10^2c.f.u/ml to 10^4c.f.u/ml resulted in significant increase in bacterial wilt severity and increase in infection response became gradual with further increasing inoculums levels. Irrespective of the different inoculums levels tested for their infection response, the bacterial wilt severity was observed higher in injured roots as compared to the uninjured roots.

The results are in agreement with the findings of Kishun and Chand (1990) [10] who reported that disease severity was maximum at inoculums levels of 10^{10} and 10^8c.f.u/ml. Similarly, Singh *et al*. (2014) [15] reported the maximum disease severity at 10^8 and 10^{10}c.f.u/ml and moderate disease severity occurred at 10^6c.f.u/ml.

Table 4: Effect of different inoculums pressure on disease development in tomato

Inoculums Conc.(c.f.u/ml)	Disease severity index (%)		Mean
	Injured roots	Uninjured roots	
10^2	64.40(54.00)	52.30(44.05)	58.21(49.02)
10^4	78.60(66.10)	71.60(57.79)	75.10(61.95)
10^6	86.60(70.13)	77.40(62.30)	82.00(66.21)
10^8	91.05(78.64)	83.20(68.82)	87.12(73.73)
10^{10}	97.00(85.60)	89.60(73.19)	93.30(79.40)
Mean	83.53(70.90)	74.82(61.23)	

$CD_{0.05}$ Inoculums Conc. (C) =1.60, Inoculation method (I) =1.01, C X I =2.45

The present results are in conformity with findings of Kishun and Chand (1990) [10] who reported highest bacterial wilt development at inoculums level of 10^{10}c.f.u/ml followed by that of 10^8c.f.u/ml. Similarly, Singh *et al*. (2014) [15] reported that maximum bacterial wilt development at 10^{10}c.f.u/ml followed by 10^8c.f.u/ml while moderate disease progression was found at 10^6c.f.u/ml.

Conclusion

Bacterial wilt caused by *Ralstonia solanacearum* is one of the devastating diseases of all solanaceous vegetables causing upto 90 percent yield losses among various crops particularly in tomato. Epidemiological studies conducted under *in vivo* conditions revealed the rapid bacterial wilt progression at higher soil temperature (30-35 °C) and soil moisture levels (90% of water holding capacity), while pH ranging between 5 to 6 was found optimum for the disease development. A significant increase in bacterial wilt development was noticed with increasing inoculums pressure from 10^2- 10^{10}c.f.u/ml. Root injury promotes the occurrence of wilt diseases.

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