Doi: HTTPS://DOI.ORG/10.23910/2/2022.0469c

# Prevalence and Epidemiology of Bacterial Wilt of Tomato

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**Article History**

Article ID: IJEP0469c
Received on 18th April, 2022
Received in revised form on 20th October, 2022
Accepted in final form on 18th November, 2022

**Abstract**

The present investigation was carried out during 2017 at the Department of Plant Pathology, Dr YS Parmar University of Horticulture and Forestry, Nauni, Solan. The objective of the study was to understand the prevalence of bacterial wilt of tomato as influenced by the edaphic factors. Periodic survey were undertaken during the crop season in major tomato growing regions and incidence of bacterial wilt of tomato ranged from 10.00% to 84.02%. 20 days old tomato seedlings were inoculated with bacterial suspension ($3x10^8$ cfu/ml) either through root dip or drenching and kept in green house with temperature 30±2°C. Inoculation of bacterial pathogen *R. solanacearum* in through seedling root dip in bacterial suspension was found better compared to drench application for development of typical symptoms of bacterial wilt disease. There was 100% disease incidence with seedling root dip method compared to 68.74% disease incidence with drenching of bacterial suspension in soil. Epidemiological studies of *R. solanacearum* revealed that with the increase in soil moisture from 40% to 90% and soil temperature from 25° to 35°, there was a progressive increase in disease incidence of bacterial wilt in tomato seedlings. Maximum disease incidence was observed at 30–35°C and at 90% soil moisture. However, no disease was observed at 20°C and at 20% and 30% soil moisture. The finding on epidemiology from the present study will be helpful to design effective disease management strategy.

***Keywords:*** Edaphic factors, artificial inoculation, epidemiology, soil-borne, solanaceous crops

## 1. Introduction

Soil-borne bacterial diseases are major constraints in the production of many economically important crops. Different management diseases management strategies including the development of new chemicals are emerging continuously (Sharma, 2016, Shridhar et al., 201), yet plant disease poses a great threat to production of crop plants. Bacterial wilt of solanaceous crops, also known as southern bacterial wilt, solanaceous wilt, southern bacterial blight, is one of the most devastating diseases in solanaceous crops. Bacterial wilt of tomato, caused by *Ralstonia solanacearum* is one of the destructive soil-borne disease (Gupta et al., 2017). The disease is of common occurrence in tropical and subtropical regions and reduces overall yield of crop. The bacterial wilt disease can cause upto 90% yield losses in tomato (Elphinstone, 2005). The bacterial wilt disease of tomato is widespread in India including the states of Karnatka, Kerala, Maharashtra, Odisha, Bihar, West Bengal and Himachal Pradesh (Gupta et al., 2017). Mondal et al. (2011) reported the incidence of bacterial wilt disease in tomato, brinjal and potato in West Bengal with 9.86% to 86.45% damage. The bacterial pathogen attaches and then colonizes the roots of host plant. Then, the bacterium invades the xylem tissues and transmits to the aerial parts of the host plant employing xylem as a route. Persistence of high bacterial load within the plant xylem and accumulation of copious amount of exo-polysaccharides (EPS) in the xylem is assumed to be the cause of the irreversible wilting of the infected plant culminating in plant death (Hikichi et al., 2017). The presence of large amounts of *R. solanacearum* cells and their EPS in xylem vessels results in reduction in sap flow leading to wilting of the host plant (Genin and Denny, 2012). Infected plants suffer yellowing, stunting, wilting and often die rapidly. Symptoms also include leaf epinasty and browning of vessels (Yahiaoui et al., 2016). Additional symptoms are water soaking of pith followed by its browning and browning of cortex near the soil line during later stages of infection (Sarkar and Chaudhuri, 2016). Older plants first show wilting of youngest leaves, or one sided wilting and stunting, and then finally the plants wilt permanently (Mandal et al., 2014). Temperature and soil moisture affect the multiplication and survival of *R. solanacearum* in many crops (Islam and Toyota, 2004, Champoiseau and Momol, 2009, Mondal et al., 2014, Wei et al., 2015). There is a very sharp relationship between intensity of wilt diseases and different meteorological factors (Singh et al., 2014, Attri et al., 2018). Temperature is the most important factor that affects host-pathogen interactions

(Gupta et al., 2022). Avoiding the development of tomato plants during the period of high environmental temperature by altering the transplanting dates helped in reducing the bacterial wilt disease incidence (Wei at al., 2015). Soil moisture also affects multiplication and survival of *R. solanacearum*. Well drained soils with higher water retention capacity are favorable for the survival of the bacterial pathogen. Wilt incidence and survival of pathogen was lower under dryer soil conditions like 20% to 30% of maximum water holding capacity of soil (Islam and Toyota, 2004).Sound knowledge on effect of soil epidemiology on disease development will be helpful to design better disease management strategies. Additionally, due to changing environmental conditions, the occurrence of plant diseases is also changing (Sharma et al., 2020). Hence, the present investigation is carried out to study the prevalence of bacterial wilt disease of tomato and the effect of edaphic factors *viz*., soil temperature and soil moisture on the disease development.

## 2. Materials and Methods

### *2.1. Prevalence of bacterial wilt*

The study was undertaken during March to July, 2017 at the Solan and Sirmaur districts of Himachal Pradesh, India. Total number of healthy and wilted plants were recorded in 2×2 $m^2$ area in ten disease prone fields of the locality and averaged for the village. The diseased plant samples along with soil from rhizosphere were also collected in paper bags and brought to laboratory for confirming the association of the bacterium and to isolate the pathogen. The disease incidence was calculated by the following formula:

Disease incidence (%) =(Total number of wilted plants/ Total number of observed plants)×100 ..... (1)

The samples showing the presence of ooze and vascular discolouration were selected for isolation. The roots of diseased plants were washed thoroughly in running tap water and small bits (2 mm) were taken from infected portion after proper sterilization with sodium hypochlorite (0.1%) solution. Then bits were added in sterilized distilled water and placed on shaker for four days. After that when suspension became translucent, loopful of bacterial cell suspension was streaked on Petri plates containing the nutrient agar medium and incubated at 28± 1°C. After 36–72 h, the Petri plates were examined and culture was further purified and multiplied on triphenyl tetrazolium chloride basic medium, which is a specific medium for the growth of *Ralstonia solanacearum*. The pathogen was identified on the basis of morphological, cultural and biochemical characters (Schaad et al., 2001).

### *2.2. Pathogenicity of bacteria*

Pathogenicity of isolated bacterial pathogen was conducted by two methods i.e. seedling root dip in bacterial suspension and drenching of bacterial suspension in soil. The lateral roots of 20 days old tomato seedlings of cultivar Solan Lalima were cut with the help of sterilized scissors in order to create injuries to the roots. The injured roots were dipped in bacterial suspension ($3 \times 10^8$ cfu $ml^{-1}$) for 30 minutes and then transplanted in pots containing sterilized soil. For inoculation of bacterial pathogen through drenching in soil, seedlings with injured roots were transplanted in soil and then pots were flooded with bacterial suspension ($3 \times 10^8$ cfu $ml^{-1}$). Plants were regularly irrigated and kept in greenhouse having temperature 30± 2°C for development of symptoms.

### *2.3. Epidemiological studies*

To conduct the epidemiological experiments, tomato seedlings were raised and inoculated with bacterial suspension ($3.0\times10^8$ cfu $ml^{-1}$) by root dip method or suspension drenching method. Three seedlings per pot were planted. They were regularly irrigated till the establishment of seedlings.

#### *2.3.1. Effect of different temperature regimes on bacterial wilt disease development*

In order to study the effect of different temperature regimes on the bacterial wilt development, an experiment was conducted in Completely Randomized Design (CRD). These pots with 20 days old tomato seedlings inoculated with bacterial suspension, either as seedling root dip or drenching, were kept at requisite temperature levels *viz*. 20, 25, 30, 35 and 40°C. Each treatment was replicated four times. The number of wilted plants at each temperature were recorded after 11 or 12 days and disease incidence was calculated.

#### *2.3.2. Effect of different soil moisture levels on bacterial wilt disease development*

In order to study the effect of different soil moisture regimes on the bacterial wilt disease development, an experiment was conducted in Completely Randomized Design (CRD). Different soil moisture regimes *viz*. 20%, 30%, 40%, 50%, 60%, 70%, 80% and 90% were obtained by maintaining the respective weights of soil by adding water (Attri et al., 2018). Firstly, the soil was sterilized and oven dried at 105°C to obtain 0% moisture. Then required amount of water was added to achieve specific moisture percentage based on soil water holding capacity. Each treatment was replicated three times. Moisture percentage was calculated by following formula:

$PW=(WS_1-WS_2)-WS_2\times100$ ..... (2)

where,

PW = moisture percentage

$WS_1$ = fresh weight of soil

$WS_2$ = dry weight of soil

These pots were kept in polyhouse and moisture level of pots was regularly maintained by adding the required amount of water to each pot. The number of wilted plants at each moisture percentage was recorded after 13 days and disease incidence was calculated.

## 3. Results and Discussion

### *3.1. Occurrence of bacterial wilt disease of tomato in Himachal*

*Pradesh*

Periodic surveys of tomato growing areas of Solan and Sirmaur districts of Himachal Pradesh during 2017 crop season revealed that the bacterial wilt disease was widespread in Solan and Sirmaur districts. The incidence of bacterial wilt ranged from 10.00% to 84.02% in different tomato growing pockets of these districts. The average maximum disease incidence (42.13%) was at Solan district followed by Sirmour (41.11%) (Table 1). In Solan district, disease incidence was maximum at Nadog (84.02%) followed by Nandal (70.45%), Mansar (45.00%) and Dihari (43.33%) in contrast to lowest incidence at Deothi (10.00%). In Sirmaur district, maximum disease incidence was at Chakhal (60.00%) followed by Gaura (54.21%) and Karganu (47.71%) in contrast to lowest incidence at Giripul (27.00%). Different biochemical tests were conducted to ascertain the identity of the pathogen and presented in Table 2. The pathogen was identified as *Ralstonia solanacearum* on the basis of morphological, cultural and biochemical characters. Bacterial wilt disease of tomato is an endemic disease in most of the districts of the state and causes 80–100% losses in heavily infested fields in the state. In Himachal Pradesh, the disease was first observed in Kangra valley in 1981 and thereafter it gradually spread to other districts i.e. Kullu, Mandi (Sood and Singh, 1993), Solan (Gupta et al., 1998), Bilaspur and Hamirpur (Sood et al., 2002). The nomenclature of the members of *R. solanacearum* species complex was revised by using a polyphasic taxonomic approach which grouped them either with *R. solanacearum*, *Ralstonia syzygii, Ralstonia syzygii* subsp. *indonesiensis*, *R. syzygii* subsp. *celebesensis* or *R. syzygii* subsp. *pseudosolanacearum* (Safni et al., 2014).

Table 1: Incidence of bacterial wilt of tomato in Solan and Sirmaur districts of Himachal Pradesh

| District/Locality | Disease incidence (%) |
|---|---|
| Solan district | |
| Deothi | 10.00 |
| Dhali | 36.86 |
| Dihari | 43.33 |
| Dolag | 33.33 |
| Jadari | 40.33 |
| Mansar | 45.00 |
| Nandal | 70.45 |
| Nauni | 29.99 |
| Nadog | 84.02 |
| Srinagar | 28.06 |
| Mean | 42.13 |
| Sirmaur district | |
| Chakhal | 60.00 |
| Giripul | 27.00 |
| Karganu | 47.71 |
| Kotla | 41.00 |
| Panjela | 43.33 |
| Ser Banera | 36.39 |
| Dhar | 33.39 |
| Gaura | 54.21 |
| Kotla | 30.00 |
| Mean | 41.44 |

Table 2: Biochemical characterization of isolated *Ralstonia solanacearum*

| Sr. No. | Tests | Reactions | Observations |
|---|---|---|---|
| 1. | Growth on triphenyl tetrazolium chloride | Positive | Fluidous colonies with white margin and pinkish centre |
| 2. | Gram staining | Gram negative | Retained the colour of safranine |
| 3. | Hydrolysis of gelatin | Negative | Medium retained in solid state |
| 4. | Starch test | Negative | No clear zone around colonies |
| 5. | Kovac's oxidase test | Positive | Blue colour development |
| 6. | Catalase test | Positive | Appearance of gas bubbles |
| 7. | Citrate utilisation test | Positive | Change in colour from green to blue |
| 8. | Nitrate reduction test | Positive | Deep red colour development |
| 9. | Utilization of sucrose | Positive | Yellow colour development and gas production |
| 10. | Utilization of glucose | Positive | Yellow colour development |

### *3.2. Pathogenicity of isolated Ralstonia solanacearum in tomato seedlings*

Pathogenicity tests of isolated *Ralstonia solanacearum* on tomato cultivar Solan Lalima by two methods of bacterial inoculation showed that there was 100% disease incidence with seedling root dip method compared to 68.74% disease incidence with drenching of bacterial suspension in soil (Table 3). Typical symptoms of bacterial wilt i.e. drooping of leaves and vascular discoloration were more prominent in seedling root dip method as compared to suspension drenching method. However, similar incubation period of 11 days and 12 days were observed with drenching of bacterial suspension in

Table 3: Pathogenicity of *Ralstonia solanacearum* in tomato

| Sr. No. | Method of inoculation | Disease incidence (%) | Vascular discolouration | Incubation period (days) |
|---|---|---|---|---|
| 1. | Seedling root dip | 100.00 | ++++* | 12 |
| 2. | Drenching | 68.74 | ++** | 11 |

++++*: More discolouration; ++**: Less discolouration

soil and with seedling root dip in bacterial suspension method, respectively. Kishun and Chand (1990) reported 9 days for symptom appearance by root dip method. Similarly, 12 days and 14 days for symptom appearance in suspension drenching and seedling root dip method, respectively were reported (Kumar, 2003). However, soil drenching method of inoculation was used to inoculate the bacterial wilt pathogen in tomato as well as in brinjal and chilli (Hanson et al., 1996, Artal et al., 2012). Although the inoculation of the bacterial pathogen was usually done of adult plants, Kumar et al (2017) and Singh et al (2018) described inoculation methods on 6-7 days old seedlings. Inoculation of *R. solanacearum* on 6 to 7 days old tomato seedlings by a simple leaf-clip strategy resulted in a lethal pathogenic condition in seedlings that eventually killed these seedlings within a week post-inoculation (Kumar et al., 2017). Roots of 6 to 7 days old tomato seedlings were dipped in the bacterial inoculum (up to the root-shoot junction) followed by transfer of the seedling to an empty 1.5- or 2.0-ml sterile microfuge tube (Singh et al., 2018). The root-dip-inoculated seedlings transferred to microfuge tubes were subjected to air exposure for approximately 5 min prior to addition of 1 to 1.5 ml of sterile water to each tube. Approximately 80 to 90% of the inoculated seedlings were found to be dead after seven day of inoculation.

### *3.3. Epidemiological studies*

#### *3.3.1. Effect of different temperatures regimes on disease incidence*

Disease incidence of bacterial wilt of tomato was studied at different soil temperature levels ranging between 20–40°C in tomato seedling inoculated with bacterial suspension ($3.0\times10^8$ cfu $ml^{-1}$) by root dip method or suspension drenching method. Observations on disease incidence after 13 days of transplanting revealed that in seedling root dip method, disease incidence was maximum (100.00%) at 30°C and 35°C temperatures and was minimum (23.33%) at 25°C whereas no disease development occurred at 20°C (Table 4). While at 40°C, none of the plant could survive due to high temperature. In drenching method where tomato seedlings were challenge inoculated with bacteria by suspension drenching, maximum disease incidence of 68.74% was observed at 30°C followed by 60.41% at 35°C (Table 3). Minimum disease incidence of 22.91% was recorded at 25°C while no disease was observed at 20°C. Higher disease incidence was observed with seedling

Table 4: Effect of different temperature regimes on development of bacterial wilt disease of tomato

| Temperature (°C) | Disease incidence (%) | |
|---|---|---|
| | Seedling root dip method | Drenching method |
| 20 | 0.00 (0.00) | 0.00 (0.00) |
| 25 | 23.33 (28.81) | 22.91 (25.11) |
| 30 | 100.00 (90.00) | 68.74 (56.10) |
| 35 | 100.00 (90.00) | 60.41 (51.15) |
| 40 | 0.00 (0.00)* | 0.00 (0.00)* |
| CD ($p$=0.05) | 1.54 | 1.41 |
| SEm± | 0.507 | 0.464 |
| SEd± | 0.718 | 0.656 |

root dip method compared to drenching method at all the temperature level. The results of present studies are in conformity with Hayward (1991) and Wang and Lin (2005) who observed that disease incidence and symptoms in bacterial wilt of tomato were maximum at 28–30°C. Fajinmi and Fajinmi (2010) clearly indicated that *R. solanacearum* causing wilt disease was most severe on plants when temperature ranged between 25°C and 35°C and its aggressiveness decreased, either above 35°C or below 18°C temperature. Results of present studies are also in confirmation with the results obtained by Champoiseau and Momol (2009) reported that temperature of 29 to 35°C was associated with an increase in severity of bacterial wilt in several hosts including tomato. The pathogen rapidly moved through the plant at ambient temperatures above 28°C. Mondal et al. (2014) recorded maximum bacterial blight disease intensity in rice plants at temperature range of 30–35°C. Results obtained from present studies indicated that optimum temperature for bacterial wilt development in tomato was 30–35°C and low and high soil temperatures were not favourable for disease development. Avoiding high temperature is helpful to reduce the incidence of bacterial wilt disease (Wei et al., 2015).

#### *3.3.2. Effect of different moisture levels on disease incidence*

The effect of different soil moisture regimes on the incidence of bacterial wilt of tomato was studied in tomato seedlings inoculated with seedling root dip methods and it was found that soil moisture had significant influence on the disease incidence when pots were maintained at various moisture regimes. It is evident from the data in Table 4 that the disease was able to develop at all soil moisture levels except at 20% and 30% where disease did not occur at all. With the increase in soil moisture level, there was a corresponding increase in disease incidence and maximum disease incidence (100.00%) was recorded at 90% soil moisture level, which was significantly different from other moisture regimes. It was followed by 80%, 70%, 60%, 50%, and 40% soil moisture levels wherein disease incidence of 82.21%, 73.33%, 66.66%, 44.44%

and 35.55%, respectively (Table 5). The results clearly indicate that higher soil moisture is favourable for the development of disease. These results are in agreement with Islam and Toyota (2004) who suggested that wilt incidence and survival of pathogen was lower under dryer soil conditions like 20% to 30% of maximum water holding capacity of soil.

Soil borne diseases are very difficult to manage due to their highly heterogeneous incidence and lack of knowledge on the epidemiological aspects of soilborne pathogens. The effective control of the soilborne diseases is possible only through detailed study on survival, dissemination of soilborne pathogens; effect of environmental conditions role of cultural practices and host resistance and susceptibility will play a major role in disease management. The biocontrol agents are used for the management of soil borne diseases (Islam et al. 2010). The effectiveness of biocontrol agents may also be influenced by the ediaphic factors of the soil. Many factors in the soil influence the activity of soilborne pathogens and diseases. Our study clearly demonstrated that the bacterial wilt of tomato is more severe in wet soils than in dry soils.

Table 5: Effect of different soil moisture regimes on development of bacterial wilt disease of tomato

| Moisture level (%) | Disease incidence (%) |
|---|---|
| 20 | 0.00 (0.00) |
| 30 | 0.00 (0.00) |
| 40 | 35.55 (36.49) |
| 50 | 44.44 (41.78) |
| 60 | 66.66 (55.00) |
| 70 | 73.33 (59.39) |
| 80 | 82.21 (65.76) |
| 90 | 100.00 (90.00) |
| CD ($p$=0.05) | 2.706 |
| SEm± | 0.922 |
| SEd± | 1.304 |

## 4. Conclusion

High disease incidence of bacterial wilt of tomato in leading tomato growing districts of Himachal Pradesh was recorded. Maximum incidence of bacterial wilt of tomato was at soil temperature of 30–35°C and soil moisture of 90%.

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