



Epidemiological assessment, isolation and pathogenicity of *Ralstonia solanacearum* species complex (RSSC) associated with bacterial wilt of tomato in Annamayya District, Andhra Pradesh, India

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Abstract

Bacterial wilt is an important disease affecting tomato production, with considerable variation in disease occurrence between locations and growing seasons. A roving survey was conducted during Kharif and Rabi 2025 to assess the occurrence, incidence and severity of bacterial wilt of tomato in major tomato-growing areas of Annamayya district, Andhra Pradesh, India. Nine villages, namely Tekkulapalem, Malepadu, Panasamakulapalli, Marumpalli, Gattu, Seethavaripalli, Tettu, Kotavooru and Angallu, were surveyed during the two cropping seasons. Bacterial wilt was observed in all surveyed areas, with disease incidence and severity varying among locations and between seasons. During Kharif, disease incidence ranged from 19.30 to 40.60%, whereas during Rabi it ranged from 10.66 to 28.00%. The highest disease incidence was recorded at Malepadu during both Kharif (40.60%) and Rabi (28.00%). Disease severity during Kharif ranged from 20 to 55%, while during Rabi it ranged from 10 to 30%. The highest severity was recorded at Malepadu, with 53.00% during Kharif and 36.00% during Rabi. The lowest incidence during Kharif was recorded at Panasamakulapalli (19.30%), while the lowest incidence during Rabi was recorded at Gattu (10.66%). The lowest disease severity was recorded at Kotavooru during Kharif (22.00%) and Angallu during Rabi (11.00%). Overall, bacterial wilt was more pronounced during Kharif than Rabi across the surveyed locations. The survey demonstrated considerable spatial and seasonal variation in bacterial wilt occurrence in tomato-growing areas of Annamayya district. The results provide baseline information for disease surveillance and development of location- and season-specific bacterial wilt management strategies.

Keywords: Bacterial wilt, tomato, disease incidence, disease severity, seasonal variation, field survey, Annamayya District, Andhra Pradesh

Introduction

Tomato (*Solanum lycopersicum* L.) is one of the most popular vegetable crops grown in the world, next to potato. Globally, tomatoes are cultivated across 5 million hectares (Mha) area with annual production of 189.1 million tons approximately. The yield losses in tomato caused by *Ralstonia solanacearum* vary from 0% to 90% depending on the cropping pattern, pathogen strain, cultivar, soil type, and climate (Nion and Toyota, 2015) [2]. In 2024-25, the country was projected to produce 215.49 lakh tonnes of tomatoes, an increase of 1.06% over the previous year. In Andhra Pradesh, Annamayya, Chittoor, Prakasam, Anantapur, Kurnool, and Visakhapatnam are the major tomato growing districts. Madanapalle division of Annamayya followed by Punganur and Palamaner divisions of Chittoor district are leading tomato cultivation and trade centers. Bacterial wilt caused by *Ralstonia solanacearum* is considered one of the most destructive diseases, leading to severe yield losses in India as well as in several other countries worldwide (Rao *et al.*, 1975) [5].

The occurrence and intensity of bacterial wilt can vary considerably between locations and growing seasons. Understanding the distribution, incidence and severity of the disease is therefore important for identifying areas with

greater disease pressure and for planning appropriate disease-management and surveillance programmes.

Annamayya district represents an important tomato-growing region of Andhra Pradesh. However, information on the field distribution and seasonal variation of bacterial wilt across tomato-growing areas of the district is limited. Therefore, a roving survey was conducted during Kharif and Rabi 2025 to document the occurrence, incidence and severity of bacterial wilt in major tomato-growing locations of Annamayya district.

Materials and Methods

Survey for incidence of tomato wilt complex

Tomato plants samples showing typical wilt symptoms were collected from tomato growing areas of Annamayya district i.e. Madanapalle, B. Kothakota, Kurabalakota mandal during Kharif, 2025 and Rabi, 2025. Fig 1

A roving survey was conducted to assess the disease incidence and disease severity where three samples from each village and three villages in each mandal was collected. Samples were collected based on the random sampling method. Diseased plants were uprooted and put into plastic ziplock covers. These samples were labelled with date of collection, variety name, place along with GPS location and taken to laboratory for further process.

Additional information on the parameters like variety name, place, crop growth stage, crops in neighbouring fields and plant parts affected were recorded and based on the positive results obtained out of total samples collected, per cent wilt incidence was calculated for bacterial using the following formula.

$$\text{Per cent disease incidence} = \frac{\text{Total number of diseased plants}}{\text{Total number of tested plants}} \times 100$$

and disease severity was calculated using the scale.

Rating scale for Bacterial wilt (Phiri et al., 2024)^[3]

- 0 (no wilting symptoms, healthy),
- 1 (0%–25% wilting leaves),
- 2 (25%–50% wilting leaves),
- 3 (50%–75% wilting leaves), and
- 4 (75%–100% wilting leaves).

$$\text{Disease severity} = \frac{\sum (\text{number of plants with corresponding disease index} \times \text{disease severity})}{\text{Total number of tested plants} \times \text{highest disease level}} \times 100$$

Isolation and Identification of *Ralstonia solanacearum*

Tomato plants exhibiting typical bacterial wilt symptoms, including vascular discoloration, were collected for pathogen isolation. Bacterial ooze from infected vascular tissues was confirmed by placing freshly cut stem pieces in sterile water. Discolored vascular tissues (4–5 mm) were surface sterilized with 1% sodium hypochlorite for 30 seconds, rinsed with sterile distilled water, and suspended in 10 mL sterile water for 10 minutes. The resulting bacterial suspension was serially diluted and 100 µL of the appropriate dilution was spread on Triphenyl Tetrazolium Chloride (TZC) agar medium (Kelman, 1954)^[1]. Plates were incubated at 32°C for 48 h. Typical virulent colonies of *R. solanacearum*, characterized by irregular, fluidal, dull-white colonies with pink centers, were selected for further studies.

Purification and Preservation of Isolates

Typical virulent colonies were purified by repeated streaking on TZC agar and incubation at 32°C for 48 h. Pure cultures were subsequently maintained in CPG broth. For long-term preservation, 1 mL of 72-h-old bacterial culture was mixed with 1 mL of sterile 40% glycerol and stored at –20°C. Additionally, glycerol stocks were prepared by suspending four loopfuls of 48-h-old pure culture in a

mixture of 1 mL sterile distilled water and 1 mL of 40% glycerol and stored at –20°C.

Pathogenicity test

Inoculum was prepared from isolates preserved in glycerol stocks. All the 27 *Ralstonia solanacearum* isolates were freshly grown in CPG-TZC media at 28°C for 48 hours. Characteristic single slimy colonies of each isolate showing exopolysaccharide secretions were re-cultured on CPG media without TZC for 48 hours at 28 ± 1 0 C. The cells were plated by serial dilution technique in CPG-TZC media and inoculum load of 10⁸ cfu/ml was prepared. Pathogenicity test was performed using host tomato (Sahoo (TO 3251)). Seedlings were prepared in green house and transplanted after 3 weeks into pots containing sterilized soil. The lateral roots of the seedlings were given injury prior transplanting. The prepared inoculum of strength 10⁸ cfu/ml was poured surrounding the root zone by the method of soil drenching. Five seedlings of each host plants were inoculated for each isolate in different pots. Control was maintained by soil drenching with sterilized double distilled water. The inoculated seedlings were watered regularly. Development of symptom was monitored after every week for 1 month.

Results

A roving survey conducted across Annamayya district during the *Kharif* and *Rabi* 2025 seasons revealed that bacterial wilt is prevalent in mild to severe forms, displaying strong seasonal variation. Overall, both disease incidence and severity were markedly higher during the warm, high-humidity *Kharif* season than the cooler *Rabi* season. (Table 1). Average disease incidence spanned 20% to 40% in *Kharif* and dropped to 15% to 28% in *Rabi* (Fig 1.). Across both seasons, Malepadu village (Madanapalle mandal) consistently recorded the highest disease levels, peaking at 40.60% incidence and 53.00% severity in *Kharif*, and 28.00% incidence and 36.00% severity in *Rabi*. Conversely, the lowest *Kharif* incidence occurred in Panasamakulapalli village (19.30%), while Gattu village recorded the lowest *Rabi* incidence (10.66%). Disease severity similarly trended lower in *Rabi* (10% to 30%) compared to *Kharif* (20% to 55%) (Fig 2.). The lowest severity shifted from Kotavooru village in *Kharif* (22.00%) to Angallu village in *Rabi* (11.00%). Isolation of the RSSC was done from infected samples of tomato collected from the survey over various mandal of Annamayya district of Andhra Pradesh. A total 27 pure cultures of *Ralstonia solanacearum* were isolated from 3 mandals of Annamayya district of AP.

Table 1: Details of different isolates collected and studied from tomato growing areas of Annamayya district of Andhra Pradesh

District	Mandal	Village	Isolate code	Variety	GPS Location	Stage of crop	Disease incidence (Kharif)	Disease incidence (Rabi)	Disease severity (Kharif)	Disease severity (Rabi)
Annamayya		Tekkulapalem	TE 1, TE 2, TE3	Champion	13.5503°N	40 Days Flowering	28.60%	16.60%	36.50%	23.50%
					78.5029°E					
	Madanapalle	Malepadu	MA1, MA2, MA3	Vaibhav	13.503913°N	Flowering	40.60%	28%	53%	36%
					78.451102°E					
		Panasamakulapalli	PA1, PA2, PA3	Ashutosh	13.57009°N	Fruiting	19.30%	18%	29%	25%
					78.44523°E					
Annamayya		Marumpalli	BK1,	Jayahoo	13.6575549°N	Flowering	27.30%	23.30%	44.50%	28.50%

			BK2, BK3		78.2625728°E					
	B. Kothakota	Gattu	GA1, GA2, GA3	Sahoo (TO 3251)	13.6575549°N 78.2625728°E	Fruiting	23.30%	10.66%	31%	13%
		Seethavaripalli	SE1, SE2, SE3	Salaar 3119	13.6575549°N 78.2625728°E	Fruiting	30%	12.60%	28.50%	17%
		Tettu	TET1, TET2, TET3	Champion	13.618523°N 78.441388°E	Fruiting	33.30%	16%	41%	21.50%
	Kurabalakota	Kotavooru	KO1, KO2, KO3	Jayahoo	13.686348°N 78.421364°E	Fruiting	20%	19.30%	22%	23%
		Angallu	AN1, AN2, AN3	Sahoo (TO 3251)	13.68632°N 78.421332°E	Flowering	26.6	13.30%	33.10%	11%

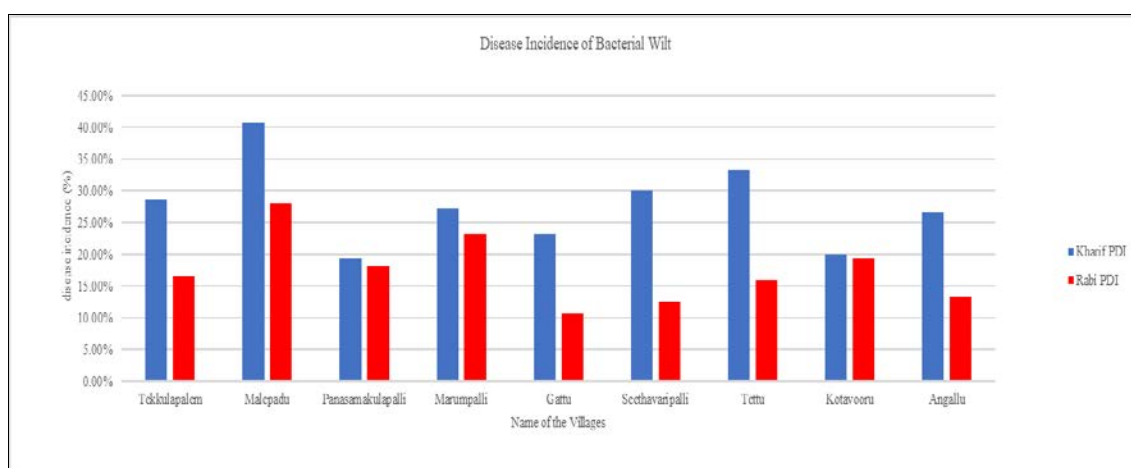


Fig 1: Prevalence of bacterial wilt disease incidence across nine villages of Annamyya district in *Kharif* 2025 and *Rabi*-2025 respectively.

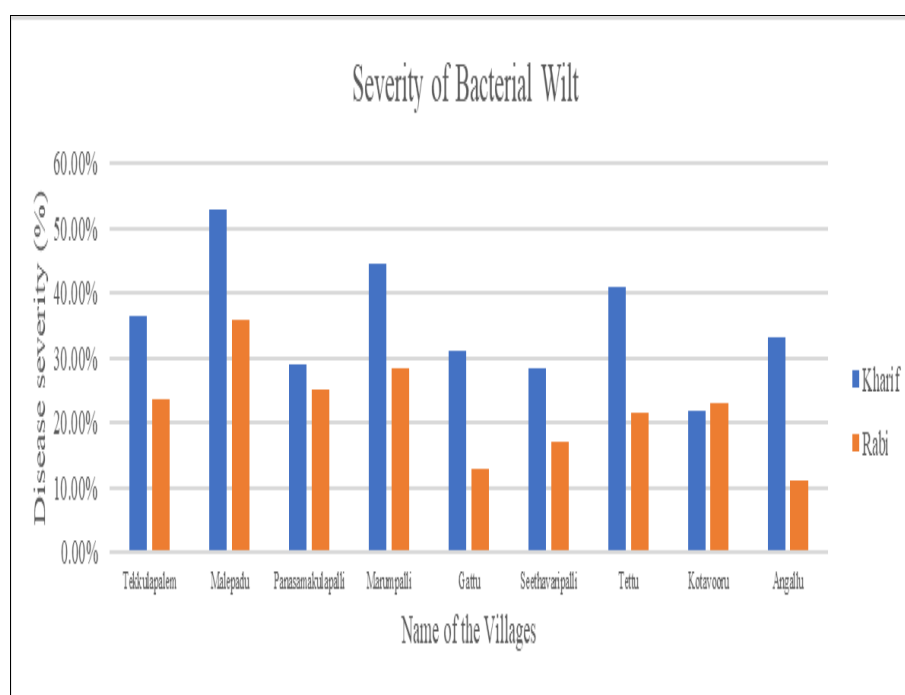


Fig 2: Prevalence of bacterial wilt disease severity across nine villages of Annamyya district in *Kharif* 2025 and *Rabi* 2025 respectively.

Isolation of the bacterial wilt-associated pathogen

The casual organism was isolated from each of infected plant exhibiting typical bacterial wilt symptoms collected from various regions of Annamyya district of Andhra

Pradesh. Isolation was done by spread plate method using triphenyl tetrazolium chloride agar (TZC) medium. Well separated bacterial colonies obtained by repeated isolation on Casamino acid Peptone Glucose Agar medium after 48

hours of incubation at 32°C yielded virulent colonies which were irregularly shaped, fluidal, dull white colonies with a

pink center and avirulent colonies were small, round, convex with large red pigment and narrow margins. (Fig 4)



Fig 3 (a-e): Symptoms of bacterial wilt disease in tomato

Pathogenicity test

Koch's postulates were carried out to confirm the pathogenic nature of collected isolates *Ralstonia solanacearum*. A total of twenty-seven bacterial isolates obtained during the study were evaluated for their pathogenicity by following the method discussed above. For the pathogenicity test, a bacterial suspension containing 5×10^8 cfu/mL of each isolate was prepared and inoculated through soil drenching onto susceptible tomato plant variety

Sahoo (TO 3251). The result showed that all the tested twenty-seven isolates caused wilt symptoms on Tomato plants. The virulence level of tested isolates demonstrated statistically significant variation between highly virulent and moderately virulent isolates at Tukey's test ($\alpha=0.05$). One of the RSSC isolate SE3 caused tomato wilt within 7 days after inoculation and was classified as highly virulent. Remaining RSSC isolates caused wilt of tomato between 7 and 15 days after inoculation and were classified as moderately virulent

Table 2: Pathogenicity and virulence of *Ralstonia solanacearum* (RSSC) isolates collected from Annamayya district of Andhra Pradesh, India on tomato plant variety Sahoo (TO 3251)

Isolates	Days of wilt symptoms observed	Pathogenicity group
TE1	12 ^e	2
TE2	11.5 ^e	2
TE3	11.5 ^e	2
MA1	11.5 ^e	2
MA2	11 ^e	2
MA3	11.2 ^e	2
PA1	13 ^e	2
PA2	12.3 ^e	2
PA3	12 ^e	2
BK1	11 ^e	2
BK2	11 ^e	2
BK3	11.5 ^e	2
GA1	10.8 ^e	2
GA2	13.0 ^e	2
GA3	10.0 ^{de}	2

SE1	10.8 ^e	2
SE2	11.5 ^e	2
SE3	7.0 ^a	1
TET1	7.6 ^{ab}	2
TET2	11 ^e	2
TET3	10.8 ^e	2
KO1	13 ^e	2
KO2	11.5 ^e	2
KO3	11.0 ^e	2
AN1	8.0 ^{bc}	2
AN2	10.0 ^{de}	2
AN3	9.0 ^{cd}	2
Control	-	-

The mean days followed by the same letters in the column are not statistically significant at Tukey's test ($\alpha=0.05$). 1 represents highly virulent isolates that cause plant wilt within 7 days of inoculation; 2 represents moderately virulent isolates that cause plant wilt between 7 and 15 days after inoculation; 3 represents weakly virulent isolates that cause plant wilt after 15 days of inoculation.

Discussion

The present survey demonstrated that bacterial wilt was widely distributed among the surveyed tomato-growing areas of Annamayya district. Disease incidence and severity varied considerably among locations, indicating spatial variation in disease pressure within the district. The present study demonstrated that bacterial wilt of tomato is widely distributed across Annamayya district, Andhra Pradesh, with significantly greater disease incidence and severity during the *Kharif* season than during *Rabi*. The higher disease pressure observed during *Kharif* is consistent with the favourable environmental conditions prevailing during this season, particularly elevated soil moisture, higher temperatures, and increased relative humidity, which enhance the survival, multiplication, and dispersal of the *Ralstonia solanacearum* species complex (RSSC) in soil and promote infection through wounded or actively growing roots. These observations agree with earlier reports describing warm and humid conditions as major drivers of bacterial wilt epidemics in tropical tomato-growing regions. The most prominent observation was the difference between the two cropping seasons. Disease incidence and severity were generally higher during *Kharif* than *Rabi*. During *Kharif*, incidence reached 40.60% at Malepadu and severity reached 53.00%, whereas the corresponding values during *Rabi* were 28.00 and 36.00%, respectively. The consistently higher disease levels observed during *Kharif* indicate that the disease was more intense during this season under the conditions prevailing during the survey period. The lower disease levels recorded during *Rabi* suggest comparatively reduced disease pressure. Considerable variation was also observed among villages. Malepadu recorded the highest disease incidence in both *Kharif* and *Rabi*, indicating that it represented the location with the greatest disease pressure among the surveyed areas. In contrast, Panamakulapalli recorded the lowest *Kharif* incidence, while Gattu recorded the lowest *Rabi* incidence. A similar location-specific pattern was observed for disease severity. Malepadu recorded the highest severity during both seasons, whereas Kotavooru recorded the lowest *Kharif* severity and Angallu the lowest *Rabi* severity. The observed variation in disease intensity demonstrates that bacterial wilt distribution was heterogeneous within the surveyed region. Differences

between locations may be associated with variation in field conditions and crop production practices; however, these factors were not specifically quantified in the present survey and therefore cannot be directly attributed as causes based on the available data.

Morphological and cultural characterization of the isolates revealed typical fluidal, irregular colonies with pink to red centres on TTC medium, consistent with the descriptions of *Ralstonia* provided by Kelman (1954), Rajan *et al.* (2002), and Singh *et al.* (2010) ^[1, 4, 6]. Artificial inoculation reproduced characteristic wilt symptoms, confirming the pathogenicity of the isolates and satisfying Koch's postulates.

The recovery of 27 pure bacterial cultures from symptomatic tomato plants further indicates the presence of the bacterial wilt-associated pathogen across the surveyed production areas. Overall, the survey establishes a baseline picture of bacterial wilt occurrence in tomato-growing areas of Annamayya district and highlights the importance of considering both location and cropping season when assessing disease pressure.

Data Availability

The disease incidence and severity data generated during the field survey are available from the corresponding author upon reasonable request.

Author Contributions

Appana Swathi: Field survey, disease assessment, sample collection, data compilation and manuscript preparation.

Jyosthna Mudigulam Karanam: Supervision, interpretation of results and manuscript review.

Viswanath Kumbharthi: Supervision and scientific review.

Karthik Reddy Panyam: Scientific inputs and manuscript review.

References

1. Kelman A. The relationship of pathogenicity in *Pseudomonas solanacearum* to colony appearance on a tetrazolium medium. *Phytopathology*, 1954;44:693–695.
2. Nion YA, Toyota K. Recent trends in control methods for bacterial wilt diseases caused by *Ralstonia solanacearum*. *Microbes and Environments*, 2015;30(1):1–11.
3. Phiri TM, Bhattarai G, Chiwina KE, Fan Q, Xiong H, Alatawi I, *et al.* An evaluation of bacterial wilt (*Ralstonia solanacearum*) resistance in a set of tomato germplasm from the United States Department of Agriculture. *Agronomy*, 2024;14(2):350.
4. Rajan PP, Gupta SR, Sarma YR, Jackson GVH. Diseases of ginger and their control with *Trichoderma*

- harzianum*. Indian Phytopathology,2002:55(2):173–177.
5. Rao MVB, Sohi HS, Tikoo SK. Reaction of wilt-resistant tomato varieties and lines to *Pseudomonas solanacearum* in India. Plant Disease Reporter,1975:59:734–736.
 6. Singh D, Sinha S, Yadav DK, Sharma JP, Srivastava DK, Lal HC, *et al.* Characterization of biovars/races of *Ralstonia solanacearum*, the incitant of bacterial wilt in solanaceous crops. Indian Phytopathology,2010:63(3):261–265.