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Insight into the prevalence, phenotypic identification and *in vitro* sensitivity assays of tomato bacterial leaf spot caused by *Xanthomonas* species in Telangana and Andhra Pradesh, India

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Abstract

Tomato bacterial spot caused by *Xanthomonas* species, a major concern leading to 22-44% yield losses and reduced market value in warm, humid conditions, prompted this study to investigate the disease incidence, phenotypic traits, and species profile of *Xanthomonas* strains in Telangana's major tomato-growing areas. The percent disease index (PDI) of BLS ranged from 14.7% to 72.5% in the surveyed tomato fields. A total of 22 *Xanthomonas* strains were isolated, of which 45.45% (10 out of 22) demonstrated the ability to utilize starch and pectin. All isolates were pathogenic to tomato and were identified as *X. euvesicatoria* through cultural, physiological and biochemical characteristics. *In vitro* evaluations of bio-control agents revealed high sensitivity of the *Xanthomonas* isolates to *Bacillus subtilis* strains. Additionally, streptomycin-resistant strains were identified, underscoring the need for effective control measures to manage the disease and prevent the spread of streptomycin resistance.

Key words: Bacterial leaf spot, tomato, *Xanthomonas* spp

Introduction

Tomato (*Solanum lycopersicum* L.; *Solanaceae* family) is ranked second most important crop among vegetable crops worldwide. India plays a significant role in global tomato production, with approximately 20.25 million metric tonnes harvested from 0.78 million ha in 2022. This makes India the second-largest producer worldwide, following China, which leads in tomato production (FAO, 2023). In India, states like Andhra Pradesh, Karnataka, Madhya Pradesh, Odisha and Maharashtra played leading roles, serving both domestic and export markets.

However, Telangana state had a significant area dedicated to tomato cultivation, spanning approximately 13, 939 ha in 2023-24 and the key districts driving this production include Rangareddy (4,628ha), Vikarabad (1,646 ha), and Siddipet (1, 228 ha) Sangareddy (877 ha), Warangal (522 ha), and Jogulamba Gadwal (457 ha) (Summer 2023-24 pre-sowing price forecast of tomato, PJTSAU). This distribution highlights the importance of tomato farming across various regions in the state.

Despite their extensive growing area, tomato production is highly susceptible to over 200 biotic stresses caused by various plant pathogens worldwide (Li *et al.*, 2023). Among bacterial diseases, bacterial leaf spot (BS) caused by multiple *Xanthomonas* species is a significant economic threat to tomato production (Osdaghi *et al.*, 2021; EPPO, 2024). Identifying the pathogens remains a major challenge for effective BS management due to frequent mutations complicating pathogen detection (Adhikari 2020). The incidence of BS is especially high in regions with elevated humidity and rainfall, causing crop losses ranging from 23% to 44% in severe cases (Osdaghi *et al.*, 2021; EPPO, 2024).

BS disease is caused by various bacterial strains within the genus *Xanthomonas*. Initially, it was attributed to *X. vesicatoria* (Doidge) Dawson, later revised to *X. campestris* pv. *Vesicatoria* (Dye, 1980). Phenotypic and phylogenetic analysis deciphered that this pathovar comprises four distinct populations, all pathogenic to tomatoes, designated as groups A, B, C, and D (Dye, 1966; Stall *et al.,* 1994; Vauterin *et al.,* 1995; Jones *et al.,* 2004). Groups A and C became *X. axonopodis* pv. *vesicatoria* (now no longer valid), while Group B was renamed *X. vesicatoria* (Vauterin *et al.,* 1995; Jones *et al.,* 2000). Subsequently, new species were identified: *X. gardneri* (Group D) in the former Yugoslavia (Sutic, 1957).

Tomato BS disease symptoms are elicited on almost all aerial plant parts, including leaves, petioles, and fruits. Symptoms of leaves are initiated by the appearance of water-soaked, angular lesions that turn brown and necrotic, occasionally forming blights. On petioles and stems, the appearance of necrotic spots enlarges into canker-like lesions, while fruit lesions begin as tiny, blister-like spots that darken and crack. Pith necrosis is associated with the presence of *X. perforans* (Aiello *et al.,* 2013).

In India, BS disease was first reported in 1948 at an agricultural farm in Pune, Maharashtra (Patel *et al.,* 1950). It has since been observed in Kerala, Karnataka, Uttar Pradesh, Himachal Pradesh, Uttarakhand, and Maharashtra (Chand *et al.,* 1994). Later, a yield loss of 40% in tomato crops was recorded due to *Xanthomonas campestris* pv. *vesicatoria* (Borkar, 1997). Kavitha and Umesha (2007) found that the incidence of BS disease ranged from 22% to 50% in major tomato-growing areas of Karnataka. Shenge *et al.* (2008) explained the disease incidence varying from less than 5% to 90% between years and fields. A 35-40% disease incidence with 40% disease severity was recorded in several fields in Nashik district, Maharashtra, during the survey in 2017 (Ajayasree and Borkar, 2018). Devi *et al.* (2017) reported the phenotypic characterization and genetic diversity of 31 BS Xanthomonad isolates from five Indian states (Himachal Pradesh, Karnataka, Uttarakhand, Tamil Nadu, and Haryana), belonging to diverse agro-climatic zones. However, the EPPO database records the presence of *X. vesicatoria* with taxonomically unverified strains(https://gd.eppo.int/taxon/XANTAV/distributionand https://gd.eppo.int/taxon/XANTVE/distribution).

Although bacterial spot (BS) of tomato and chilli has been reported in several locations of India, no study or report is available on the distribution, incidence, pathogen characterization, and management in Telangana or Andhra Pradesh. Therefore, the present study was carried out to investigate the distribution and prevalence of BS disease in various tomato-growing areas of Telangana and Madanapalle, Andhra Pradesh, during 2022-2024. The study also aimed to characterize the pathogen and assess the efficacy of antagonistic bacterial bio-agents and bactericides against BS Xanthomonad isolates under *in vitro* conditions.

Materials and methods

Prevalence and collection of BS infected sample: BS disease symptoms were recorded during an extensive survey among ten districts across the three agro-climatic zones of Telangana state and four different places of Annamayya district, Andhra Pradesh during *Kharif* 2023-24. The PDI (percent disease incidence) of three random rows in each field and three fields were considered per location (Devi *et al.,* 2017). The typical BS symptomatic samples were collected in polythene bags.

Isolation, pathogenicity on tomato, hypersensitivity on tobacco and biochemical profile of BS isolates: The infective bacterium was isolated on NA medium from infected samples collected during the survey after sterilizing with 1% NaOCl and 70% ethanol followed by proper washing in distilled water according to given procedure (Schaad *et al.,* 2001). The Petri plates were incubated in BOD incubator at 27 °C for 48 h. The 48 h of pure culture of all isolates were stored separately in slants and 50% glycerol at -80 °C short- and long-term periods, respectively (Schaad *et al.,* 2001). A pathogenicity test was conducted on 4-week-old tomato seedlings to evaluate Koch's postulates of pathogens (Devi *et al.,* 2017). The tomato cv Sahoo was used for experiment. The inoculum was adjusted to approximately 1×10^8CFU. and sprayed on the leaves with a hand atomizer. Control plants were sprayed with sterile distilled water. The inoculated plants were then covered with transparent polythene covers to maintain the humidity for 2 d. Later on, daily observations were noted for symptom development. To understand the pathogenic nature of the isolates, a hypersensitive reaction test was performed on a non-host plant (tobacco) by the leaf infiltration method (Klement *et al*., 1964). *Xanthomonas* isolates suspension infiltrated large numbers (1×10^8 CFU. mL^{-1}) into the tobacco, elicited a hypersensitive response and observed tissue necrosis after 12- 24 h of inoculation. The pathogenic bacterial isolates were subjected to different biochemical tests *viz*., Gram reaction, 3% KOH, starch hydrolysis, pectin utilization test, salt tolerance test and temperature sensitivity test with three replications for further identification (Schaad *et al.,* 2001).

***In vitro* evaluation of different bio-agents against *Xanthomonas* species:** The antagonistic potential of biocontrol agents *viz*., *Bacillus subtilis, Pseudomonas fluorescens* against BS xanthomonads was achieved by conducting *in vitro* lawn spot assay with wells. Biocontrol agents were collected from the Division of Plant Pathology, ICAR-IARI (New Delhi) and the Department of Plant Pathology, PJTSAU, Hyderabad. The 100 µl of 48 h fully grown BS isolate was uniformly spread on the petri plate of King's B media. A 5 mm diameter well was made with the help of a sterilized cork borer and 100 µl of the nutrient broth of biocontrol agents at a concentration of 10^6 CFU. mL^{-1} was poured in that well. A positive control *X. euvesicatoria* XE-1 isolate and distilled water as negative control were maintained. The zone of inhibition (mm) was recorded after 3 days of incubation, and the area of inhibition was calculated using the formula πr^2 (Singh *et al.,* 2016). The experiment was repeated thrice with three replications for each treatment.

***In vitro* evaluation of streptomycin efficacy against BS xanthomonad isolates:** An *in vitro* assay was conducted to determine the inhibition efficacy of streptomycin against different BS isolates. The 48-h old fully grown BS xanthomonad isolates bacterial suspension of ($1X10^6$ CFU. mL^{-1}) was uniformly spread over a petri plate with 20 mL of the KB medium with the help of sterilized glass spreaders. Wells were punched with the use of sterilized cork borer. The effect of tested antibiotics was measured in the form of an inhibition zone surrounding the punchers by using the formula πr^2 according to Singh *et al.* (2016). The experiment was repeated thrice with three replications for each treatment.

Results and discussion

Disease incidence of tomato BLS: A total of 22 locations were surveyed in major tomato-growing regions of Telangana and Andhra Pradesh represented in Fig. 1. The typical BS symptoms like tiny brown to black spots with the yellow halo on leaves were observed and the black spots recorded on petals and young fruits. The prevalence of BLS disease in major tomato growing areas of Telangana and Andhra Pradesh along with variety is depicted in Table 1. The range of percent disease incidence was recorded from 72.5-14.7% and it was varied by location; the high BLS PDI (72.5 %) was recorded in Medak followed by 68.2 % in Adilabad, Telangana state. The lowest PDI (14.7 %) was recorded at Katlatapalli village in Annamayya district, Andhra Pradesh, followed closely by 15.9 % in another village

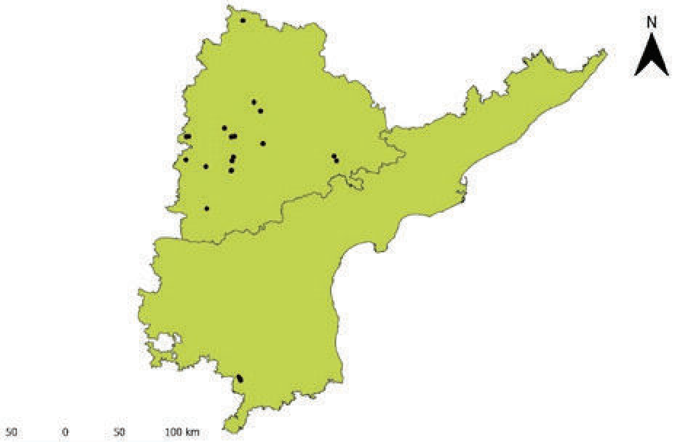

Fig.1. The map showing survey locations covered during the survey for BS disease on tomato

Table 1. The prevalence of BLS infection on tomato recorded during the survey

S.No	Sample Code	Name of the location	District	State	Latitude	Longitude	RH (%)	Temperature (°C)	Variety	Mean PDI
1	MD	Thuniki	Medak	Telangana	17.815275	78.244084	44	33	Saho (TO-3251)	72.5
2	AB	Adilabad	Adilabad	Telangana	17.649120	76.557641	48	30	Saho (TO-3251)	68.2
3	SP	Shubanpur	Rangareddy	Telangana	17.093954	78.353317	60	28	Sweakar- 448	52.4
4	SB	Shamshabad	Rangareddy	Telangana	17.261603	78.370079	54	25	Sweakar- 448	48.6
5	SP	Shankarpally	Rangareddy	Telangana	17.101262	78.360039	59	28	Sweakar- 448	50.8
6	RJ	Rajendranagar	Hyderabad	Telangana	17.328181	78.391958	52	27	Sweakar- 448	35.9
7	KK	Kanukunta	Sangareddy	Telangana	17.681232	78.411015	46	30	US 440	38.2
8	RO	Ranjole	Sangareddy	Telangana	17.674634	77.590535	49	28	US 440	35.6
9	ZB	Zaheerabad	Sangareddy	Telangana	17.677894	77.636301	42	31	US 440	18.1
10	GD	Gummidadala	Sangareddy	Telangana	17.671051	78.359292	41	32	US 440	17.5
11	PD	Pudur	Vikarabad	Telangana	17.277636	77.584279	58	22	Sweakar- 448	47.3
12	VB	Vikarabad	Vikarabad	Telangana	17.163503	77.924544	56	23	Saho (TO-3251)	41.8
13	BG	Basuvapur	YadadriBhuvanagiri	Telangana	17.551972	78.896995	49	30	Unknown	47.3
14	KM	Gudurpadu	Khammam	Telangana	17.341852	80.104994	45	33	Sweakar- 448	26.2
15	BK	Nagaram	BhadradiKothagudem	Telangana	17.260146	80.148272	45	32	Sweakar- 448	25.2
16	MJ	Mojerla	Wanaparthy	Telangana	16.448172	77.941275	41	35	Sweakar- 448	35.8
17	SC	Siricilla	Rajanna Siricilla	Telangana	18.2608063	78.7437195	54	27	Sweakar- 448	39.7
18	SP	Siddipet	Siddipet	Telangana	18.1052635	78.8576239	53	28	Unknown	41.5
19	MP1	Errasani	Annamayya	AP	13.5662780	78.5040830	49	28	Saho (TO-3251)	39.4
20	MP2	Madanapalle	Annamayya	AP	13.5264492	78.5182690	43	34	Saho (TO-3251)	41.5
21	MP3	Madanapalle	Annamayya	AP	13.5713138	78.4975418	44	33	Unknown	15.9
22	MP4	Katlatapalli	Annamayya	AP	13.5901507	78.4809693	42	34	Saho (TO-3251)	14.7

(Nimmanapalle) of the same district. In Telangana State, the lowest disease incidence (17.5) was recorded in Gummidadala, Sanga Reddy district, followed by 18.1 % in the same district. During the survey, a high temperature (35°C) was recorded at Mojerla, Wanaparthy, while a low temperature (22-23 °C) was observed in Vikarabad, Telangana. All surveyed locations recorded relative humidity above 40 % (Table 1). However, no correlation was found between disease incidence and temperature or relative humidity among the surveyed locations.Till now it was recorded in the major tomato and pepper growing regions of different states *viz*., Punjab, Haryana, Delhi, Himachal Pradesh, Uttarakhand, Karnataka, Tamil Nadu, and Kerala states (Chand *et al.,* 1994; Kavitha and Umesha, 2007; Devi *et al.*, 2017).In 2017, 35-40% BLS disease incidence with 40% disease severity was recorded in several fields in Nashik district, Maharashtra (Ajayasree and Borkar, 2018). Bacterial leaf spot on tomatoes and chilli has also been reported in various states of India including Karnataka, Tamil Nadu Kerala, Punjab, Haryana, Delhi, Himachal Pradesh, Uttarakhand(Chand *et al.*, 1994; Kavitha and Umesha, 2007; Mirik *et al.*, 2008; Shenge *et al.,* 2008; Devi *et al.*, 2017; Lokesh Babu, 2023).

Isolation, colony characteristics, pathogenicity test and hypersensitivity response (HR): A total of twenty-two bacterial isolates yielded yellow, round, mucoid, raised colonies on the NA medium as previously reported by Schaad *et al.*(2001) and Devi *et al.* (2017). The maximum colony diameter (1540µm) was observed in isolate which was isolated from samples collected in Nagaramvillage, Bhadradi Kothagudem district after 48h while, while the minimum colony diameter was 312µm recorded in isolate VB from samples collected in Vikarabad (Table 2). Several studies have observed similar colony morphology for the *X. euvesicatoria* pathogen (Patel *et al.,* 1950; Chand *et al.,* 1994).

For the pathogenicity test, all isolates of Xanthomonads isolated from tomato were inoculated on 4-week-old tomato plants of cv. Sahoo (TO-3251) using pin prick method and spray-inoculated methods. All 22 isolates showed typical disease symptoms after 5 d, with necrotic lesions surrounded by a yellow halo appearing within 2 weeks of inoculation as previously described by Devi *et al.* (2017). These symptoms are characteristic of bacterial leaf spots, with necrotic lesions surrounded by a yellow halo appearing at the infection site (Devi *et al.*, 2017; Lokesh Babu. 2023). No symptoms were observed on plants inoculated with sterile distilled water, which served as control. A hypersensitivity response (HR) was observed within 24 h of infiltration with bacterial cells on tobacco leaves, as described by Klement *et al.* (1990). Based on colony morphology, Koch's postulates and HR-positive response**,** these isolates were confirmed to be *Xanthomonas* spp infecting tomatoes (Jones *et al.,* 2004).

Biochemical and physiological assays: All 22 isolates were rod-shaped, Gram-negative and positive for 3% KOH solubility test. Among the 22 isolates, 12 isolates showed negative reactions for both starch hydrolysis and pectin utilization while 10 (45.45%) isolates (RJ, KK, BG, KM, BK, SC, SP, MP2, MP3, and MP4) showed strong positive reaction for both starch and pectin utilization assays which were characterized into two groups (Table 2). Similar kinds of atypical *X. euvesicatoria* strains with strong starch and pectin utilization were reported from India and Australia (Devi *et al.,* 2017; Roach *et al.,* 2018; Lokesh Babu, 2023). The salt stress sensitivity assay of BS xanthomonad isolates was calibrated based on bacterial growth by spectrophotometer and yielded results showing that 81.82% (18 strains) of isolates were able to tolerate the 5% NaCl while few strains 18.2% (four isolates) were retarded by 5% NaCl, being more sensitive to the salt concentration (Table 2). BLS xanthomonads could tolerate the 5% salt concentration after 72 hours of incubation. Most of the isolates were able to grow at 5% NaCl whereas only a few isolates cannot. This kind of result was reported by Haile *et al.* (2019). The growth of BLS xanthomonad isolates at 37°C was calculated after 48 h of incubation in BOD. There was a variation in the growth of bacteria at 37°C as the temperature varied from the normal recommended temperature of 25-30°C. The 13 isolates (59.09%) showed luxuriant bacterial growth in thermal stress at 37°C (Table 2). This variation among the isolates might be due

Table 2. Colony growth characteristics along with biochemical and physiological profile of *Xanthomonas* isolates

S. No	Sample ID	48 h (µm)	Catalase test	Starch hydrolysis	Pectin Utilization	Growth in OD value at 5% Nacl	Growth in OD value at 5% Nacl	Growth at 37°C
1	MD	505	+	-	-	0.424	0.083	+
2	AB	885	+	-	-	0.010	0.885	+
3	SP	596	+	-	-	0.394	1.465	+
4	SB	596	+	-	-	0.065	0.087	-
5	SP	1082	+	-	-	0.007	0.037	-
6	RJ	691	+	+	+	0.096	0.666	+
7	KK	640	+	+	+	0.201	0.320	-
8	RO	882	+	-	-	0.035	1.240	+
9	ZB	532	+	-	-	0.076	0.625	+
10	GD	562	+	-	-	0.412	0.896	+
11	PD	885	+	-	-	0.914	1.504	+
12	VB	312	+	-	-	0.241	0.821	+
13	BG	1417	+	+	+	0.080	1.565	-
14	KM	748	+	+	+	0.869	1.206	+
15	BK	1540	+	+	+	0.062	0.897	-
16	MJ	591	+	-	-	0.082	1.218	+
17	SC	513	+	+	+	0.044	1.014	-
18	SP	782	+	+	+	0.058	1.190	+
19	MP1	572	+	-	-	0.011	0.020	+
20	MP2	788	+	+	+	0.148	0.857	-
21	MP3	348	+	+	+	0.300	0.820	-
22	MP4	435	+	+	+	0.017	0.658	-
	CD(0.05)	39.68				0.0614	0.0342	
	SE(m)	13.92				0.0057	0.0120	
	CV	3.335				4.7139	2.5316	

'+' means positive reaction; '-'means negative reaction

to genetic diversity. These results were earlier reported by Haile *et al.* (2019). Till now, *the X. euvesicatoria, X. vesicatoria* and atypical strains have been reported from India.

Based on cultural characteristics observed on NA and King's B media, along with pathogenic reactions and biochemical tests, including variable responses to starch and pectin utilization, and growth at 5% NaCl, all bacterial isolates were identified as belonging to *Xanthomonas euvesicatoria.*

***In vitro* evaluation of sensitivity to different bactericides and bacterial bio-agents against *Xanthomonas* species:** *In vitro* efficacy assay of biocontrol agents against BS xanthomonad isolates showed that the range of inhibition zone of BS xanthomonad isolates recorded 38.9-0.1 cm^2 and 53.64-0.51 cm^2 by biocontrol strains *B. substilis* DTBS-5 and *B. substilis,* respectively (Table 3). Similarly, the range of the inhibition zone of BS xanthomonad isolates recorded 10.63-0.03 cm^2 by *P. fluorescens* DCPF-5. The *B. substilis* DTBS-5 strain showed significant variation in the inhibition zone over the *B. substilis* strain. In addition, the *P. fluorescens* DCPF-5 showed less sensitivity than both strains *B. substilis* DTBS-5 and *B. substilis.* Similar findings were reported by Apet *et al.* (2018). The efficacy of these agents varies based on the hydrolyzing enzymes and secondary metabolites they produce, which help prevent disease development (Leisinger and Margraff, 1979; Kharayat and Singh, 2013; Chen *et al.,* 2006; Shaikh *et al.,* 2014; Caulier *et al.,* 2019). The streptomycin preparations are most promising, which suppress the proliferation of bacteria by interacting with ribosomes, inhibiting protein synthesis at the stage of initiation of translation (Schluenzen *et al.,* 2006).

Table 3. Sensitivity of BS xanthomonad isolates against biocontrol agents after 24 h under *in vitro* conditions

S. No	Isolate	Inhibition zone (cm^2) of BS xanthomonad isolates		
		B. substilis DTBS-5	*P. fluorescens* DCPF-5	*B. substilis*
1	MD	0.1(1.81)^r	0.28	1.54
2	AB	0.2	0.12	48.25
3	SP	0.1	0.78	7.84
4	SB	0.8	1.51	0.51
5	SK	2.5	0.12	1.53
6	RJ	38.9	1.58	14.69
7	KK	3.2	0.28	4.52
8	RO	0.3	0.03	0.78
9	ZB	0.1	0.81	0.51
10	GD	1.5	0.03	3.14
11	PD	0.7	0.29	4.52
12	VB	0.1	0.03	2.54
13	BG	0.1	0.51	9.07
14	KM	0.3	0.12	6.15
15	BK	0.5	1.98	21.61
16	MJ	1.1	0.03	13.41
17	SC	8.1	0.28	11.34
18	SP	26.4	0.51	53.64
19	MP-1	12.5	0.03	26.41
20	MP-2	30.8	0.12	30.18
21	MP-3	9.1	3.71	51.94
22	MP-4	16.8	0.03	25.23
23	*X. euvesicatoria* XE1	4.4	10.63	4.93
24	CD (0.05)	0.63	0.56	0.03
25	SE(m)	0.23	0.21	0.12
26	CV	2.57	4.9	2.01

Arc sin values given in bracket; DMRT values given in superscript.

Antibiotic sensitivity of *Xanthomonas* species isolates: *In vitro* sensitivity assay of BS xanthomonads against the streptomycin of 100 ppm revealed that a range of inhibition zone exhibited by BS xanthomonad isolates 1.32-0.00 cm^2. The isolate KM showed high sensitivity and the isolates SK, RJ, BK, MP-1, MP-2, and MP-4 showed less sensitivity to antibiotic streptomycin sulphate (Table 4 and Fig. 3).

Antibiotics offer key benefits in agriculture: they penetrate plant tissues through roots, stems, and leaves, and are metabolized in seeds, providing protection independent of climate. Their antibacterial action is effective and long-lasting, and they are generally non-toxic to plants (Kolomiiets *et al.,* 2019). The streptomycin preparations are the most promising, which suppress the proliferation of bacteria by interacting with ribosomes, inhibiting protein synthesis at the stage of initiation of translation (Schluenzen *et al.,* 2006). Our study also identified

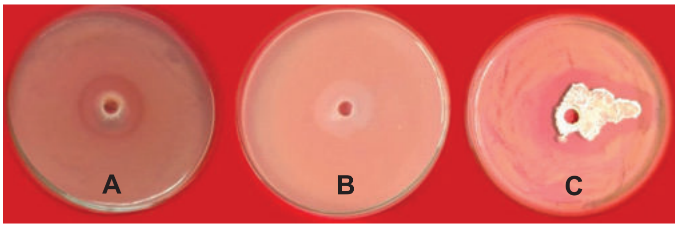

Fig. 2. *In vitro* sensitivity of BS xanthomonad isolate with biocontrol agents A) *B. substilis* DTBS-5, B) *P. flourescens* DCPF-5, C) *B. substilis* (PJTSAU).

Table 4. *In vitro* efficacy of streptomycin against *Xanthomonas* spp. isolates

S.No.	Isolate	Sensitivity to 100 ppm streptomycin
1	MD	0.29
2	AB	0.21
3	SP	0.28
4	SB	0.78
5	SK	0.03
6	RJ	0.03
7	KK	0.79
8	RO	0.19
9	ZB	0.51
10	GD	0.28
11	PD	0.19
12	VB	0.78
13	BG	0.28
14	KM	1.32
15	BK	0.03
16	MJ	0.63
17	SC	0.28
18	SP	0.63
19	MP-1	0.03
20	MP-2	0.00
21	MP-3	0.12
22	MP-4	0.00
23	Positive control	0.51
24	CD(0.05)	0.02
25	SE(m)	0.01
26	CV	4.91

Arc sin values are given in parenthesis; DMRT values are given in superscript.

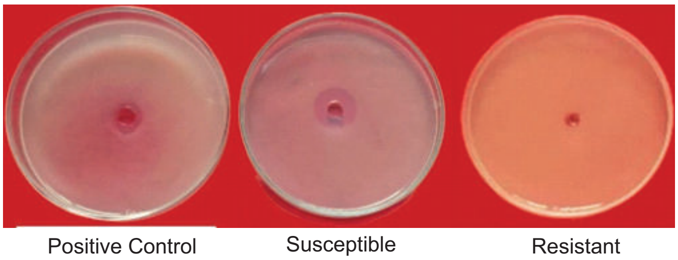

Fig. 3. *In vitro* efficacy of streptomycin against *Xanthomonas* spp. a) Positive control isolate b) *Xanthomonas* isolate showing sensitivity to streptomycin c) *Xanthomonas* isolate showing resistance to streptomycin.

the streptomycin-resistant strains of *X. euvesicatoria* (BS MP-1, BS MP-2, and BS MP-4) in Telangana and Andhra Pradesh. Similar kinds of streptomycin-resistant strains (SmR) were also recorded among phytopathogenic bacteria *E. carotovora, P. chichorii, P. lachrymans, P. syringaepv. papulans, P. syringaepv. syringee*, and *X. dieffenbachiae*. These findings highlight that streptomycin-resistant strains become a potential threat to the environment and humans by spreading antibiotic-resistant genes (McManus *et al.,* 2002).

This study revealed the widespread distribution of bacterial leaf spot (BLS) disease in major tomato-growing areas across Telangana, highlighting it as an emerging threat, particularly in warm and humid climates. Consistent with reports from other states in India, the tomato fields in Telangana exhibited significant disease incidence. The strong starch and pectin utilization traits observed in all 22 *X. euvesicatoria* isolates suggest ongoing evolutionary adaptations at the species level. Additionally, the study demonstrated the efficacy of streptomycin and biocontrol agents against BLS xanthomonad isolates, with *Bacillus subtilis* proving to be the most effective in inhibiting pathogen growth. These findings are crucial for developing integrated disease management practices aimed at controlling the spread of BLS disease in tomato crops.

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