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Seed Treatments for Management of Bacterial Canker in Tomato

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ABSTRACT: Tomato (*Lycopersicon esculentum*) is one of the important vegetable because of its high nutritive value. However, the productivity remains restricted due to a number of seed borne pathogens. Bacterial canker is one of several bacterial diseases that can seriously affect tomato plants which is caused by *Clavibacter michiganensis* subsp. *Michiganensis*, a seed borne pathogen. Seed-disseminated phytopathogens exemplify the adaptive nature of parasites, including the causal agent of bacterial canker of tomato. Hence the seed health management is an important aspect in tomato cultivation. The objective of the present study was to compare the effectiveness of copper-based compounds, antibiotics and biocontrol agents (*Trichoderma* and *Pseudomonas* spp.) in reducing the populations and spread of *C. michiganensis* subsp. *michiganensis*. In the *in vitro* studies, Streptomycin sulphate was found to be the most effective treatment amongst the chemicals screened showing a zone of inhibition of 2.33cm dia. whereas amongst the biocontrol agent screened, Pant biocontrol agent-1 (PBAT-1), a *T. harzianum* isolate was found to be the most effective showing 89.5% growth inhibition.

Keywords: Tomato, Bacterial canker, *C. michiganensis* subsp. *michiganensis*, Population dynamics, Pathogen spread, Pathogen management

The tomato *(Lycopersicon esculentum* Mill.), belonging to family Solanaceae, is an economically important crop grown throughout the world and used as both fresh and processed food. The crop is grown in a wide range of climatic conditions both in the open field and under protected conditions of cultivation [1]. However, the crop, especially in the hilly region of northern India, is susceptible to pests and diseases caused by fungi, bacteria and viruses which adversely affect the fruit quality and ultimately the supply of the fruits to the processing industries. Amongst bacterial diseases, that are frequently adding to the growers' losses are bacterial wilt (*Ralstonia solanacearum*) and bacterial spot (*Xanthomonas campestris* pv. *vesicatoria*) [2]. A new disease, bacterial canker caused by *Clavibacter michiganensis* ssp. *michiganensis* (*Cmm*) (Smith) Davis causing leaf necrosis, wilting and splitting has been reported since last few years causing crop damages in the northern hill region. The disease causes both qualitative and quantitative losses both in protected and open field cultivation [3-5]. The pathogen persists in plant debris in soil and on contaminated seeds which may act as the source of long distance dispersal of bacterial inoculum [6, 7]. The seedlings raised from *Cmm* infected seeds may be asymptomatic [8], eventually leading to the spread of the disease under field conditions. The management of the disease is also difficult, once the disease initiates, because adequate control measures are not yet available [9]. Furthermore, no resistant cultivars against the pathogen have yet been identified. However, a decline up to some extent in the pathogen spread, through antimicrobial compounds has been reported [6, 10].The present study aims on screening of chemicals, botanicals and biocontrol agents against the test pathogen under *in-vitro* conditions.

MATERIALS AND METHODS

Bacterial Culture and Growth Conditions

Through a survey carried out in tomato growing regions of Uttarakhand and Himachal Pradesh, three strains (*Cmm*5, *Cmm*6 and *Cmm*10) of *C. michiganensis* subsp. *michiganensis* were isolated, identified and subsequently sub cultured on sterilized Nutrient broth – glucose-yeast medium (NGY) (Nutrient Broth: 8.0g, Yeast extract: 2.0g, K_2HPO_4:2.0g, KH_2PO_4: 0.5g, Glucose: 2.5g, Agar: 15.0g, in 1L of distilled water followed by sterilization at 121°C, at 15psi for 15-20 min). These strains were stored in NGY medium at 4°C and were used as the working

culture while for long duration storage the bacterial cultures were kept in glycerol stock at -80°C.

In-vitro Assays

The antagonistic potentials of nine isolates of *Trichoderma harzianum* (PBAT-1, B1, B2, B3, B4, B5, B6, B7, A1, one strain of *Pseudomonas fluorescens* (PBAT-2) and one consortium of bacterial and fungal biocontrol agents (PBAT-3), four chemical compounds *viz.*, Bronopol (BP), Streptomycin sulphate (SS), Copper Sulphate(CS) and Copper oxychloride (COC) singly and in combinations of Copper Sulphate + Bronopol (CS+BP), Copper sulphate + Streptomycin sulphate (CS+SS), Copper oxychloride + Bronopol (COC+BP) and two botanicals *viz.*, neem oil and clove oil were evaluated against three strains of *C. michiganensis* subsp. *michiganensis viz.*, *Cmm* 5, *Cmm* 6 and *Cmm* 10.

The efficacy of biological treatment was determined under laboratory conditions using dual culture method [11]. Four different concentrations of chemical compounds in ppm (100, 200, 400, and 600 prepared in sterilized distilled water) were assayed under laboratory conditions using disk diffusion assay by Kirby–Bauer and Stoke´s method [12] Sterilized distilled water was used as check. The media was seeded with an initial bacterial inoculum of $1x10^8$cfu/mL. The bacterial population was monitored in both the dual culture and disk diffusion assay up to 72 hrs of plating.

Seed Inoculation and Treatment with Chemicals and Bio-control Agents

Amongst the three strains, the most virulent strain (*Cmm* 10) was sub-cultured on D_2ANX medium, the most suitable semi-selective medium, for the growth of the bacterium [13]. Bacterial growth was harvested after 48h of incubation at 28°C, and suspended in 0.1 mol/L phosphate buffered saline (PBS), pH 7.0. The inoculum density at 10^8 colony forming units (cfu/ml) was maintained by adjusting the bacterial cell suspension to 0.06 OD value in the spectrophotometer (the bacterial suspension was diluted by adding sterilized water) at the wavelength of 660 nm. The bacterial suspension was prepared for inoculation of the seeds. Seeds of the tomato variety Arka Vikas were packed (50 g) in a muslin cloth bag and placed in a 500 ml flask (Borosil) containing 200 ml of the bacterial suspension of 10^8 (cfu/ml). The seeds were left in the bacterial cell suspension for 30 min. Later, the excessive bacterial suspension was removed through the vacuum suction cups and suction pressure. The seeds were subsequently dried under aseptic conditions and treated with best effective concentration of the chemicals and biocontrol agents just before sowing.

Plant Materials and Growth Conditions

The screening of the chemical compounds and bio-control agents, under protected conditions at 28±1°C and around 65-80% RH, under glass house conditions was carried out using the variety Arka Vikas susceptible to bacterial canker and wilt diseases. The seeds treated with bacterial suspension followed by treatment with different chemicals were sown in pots (15x10 cm) on sterilized soil+ sand+ vermicompost mixture (2:1:1) under glasshouse conditions. The germinated seedlings were examined for the disease symptoms expression starting at 7 days and further at 7 days intervals up to 6 weeks.

Disease Assessment

Symptoms caused by *Cmm* in tomato plants were evaluated one week after germination under glass house and 4 weeks after inoculation in the open field screening (Table 1). Evaluation for disease appearance and subsequent development was determined using a 0-/5 arbitrary scale [14].

Table 1. Disease assessment scale

Rating score	Description
0	No leaves showing wilting
1	Slight marginal wilting, 1-/10% of leaves with wilt
2	11-/25% of leaves with wilt
3	Sectored wilting, 26-/49% of leaves showing wilting associated with chlorosis
4	Pronounced leaf collapse, 50-/74% of leaves showing wilting
5	Whole leaf wilted

Statistical Analysis

Analysis of variance (ANOVA) was performed using STPR software package versions 2 and 3, where significance level of 0.05 was used for all statistical interpretation.

RESULTS AND DISCUSSION

Symptomatology and Morphological Characteristics

The bacterial canker caused by *Clavibacter michiganensis* subsp. *michiganesis* is one of the most destructive diseases of tomato. The disease is generally

most pronounced during the months of May- August, where high temperature is accompanied by rainfall. The pathogen exhibits various symptoms on different plant parts starting from seed to fruits. The disease exhibits cankerous growth on stem and fruits and marginal necrosis in the leaves, unilateral wilting in the leaves, eventually leads to wilting of the entire plant.

The symptom of the disease becomes visible on the stem region as cankerous lesion, which increases in size chronically (A), infection on the stem often girdles the stem and may cause premature plant death. Splitting the stem longitudinally, a mealy appearance and brown colored discoloration of the internal tissues can be observed (B). The symptom on the leaves appears in the form of unilateral wilting in plant, that appears starting from the top most part and follows downside (C), marginal necrosis in leaves is also observed accompanied with downward turning of one or a few of the leaves occurs as the infection progresses (D), bird's eye spot formation in unripe and mature fruit (E, F) and growth characteristics of the bacterium in D_2ANX (G), NGY (H) medium. Curling of the oldest infected leaves followed by marginal chlorosis followed by necrosis is observed in plants [Plate1] [15].

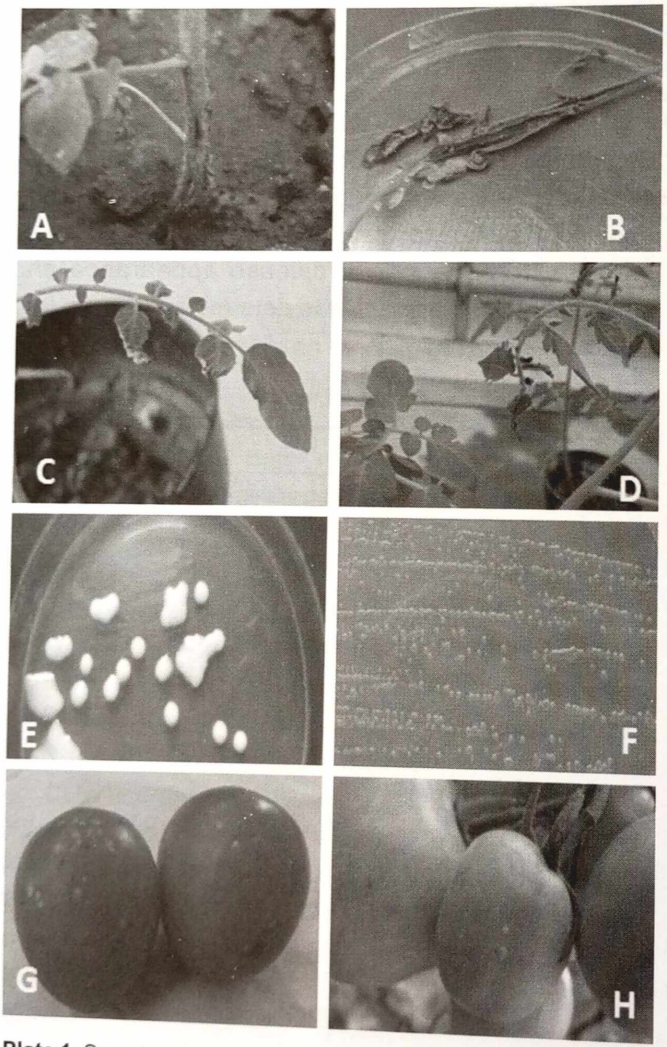

Plate 1. Symptomatology and morphological characteristics (A-H) of the *Cmm* 10 isolate A. Cankerous lesion on stem region, B. Brown colored discoloration of the internal tissues, C. Unilateral wilting in plant, D. Downward turning of the leaves, E & F. Bird's eye spot formation in unripe and mature fruit (E, F), G&H. Growth characteristics of the bacterium in D_2ANX (G), NGY (H) medium

Assessment of Chemical Compounds, Botanicals and Bio-control Agents

In-vitro assays of chemicals and botanicals

The zone of inhibition of the bacterial inoculum was taken as the indicator of the efficacy of the treatments (Table 2). The diameter of zone of inhibition differed significantly in all the treatments from control, (untreated medium showing only bacterial growth). In sensitivity test of *Cmm* to chemicals and botanicals tested, Streptomycin sulphate was found to be the most effective, followed by the combination of Copper Sulphate + Bronopol (CS+BP). Maximum (2.33cm) zone of inhibition was

Table 2. Screening of chemicals and botanicals against *Cmm*10 isolate for zone of inhibition (Disk diffusion assay)

Treatment	Concentration (ppm)	Zone of inhibition (Diameter in cm*)
Copper sulphate (CS)	100	0.0
	200	0.18
	400	0.50
	600	0.81
Bronopol (BP)	100	0.66
	200	1.21
	400	1.59
	600	1.83
Streptomycin sulphate (SS)	100	1.01
	200	1.54
	400	2.01
	600	2.33
Copper sulphate+ (CS+BP)	100	1.01
	200	1.25
	400	1.50
	600	1.83
Copper oxy chloride (COC)	100	0.46
	200	0.63
	400	0.95
	600	1.21
Copper oxy chloride+ Bronopol (COC+BP)	100	0.30
	200	0.50
	400	0.77
	600	0.89
Neem Oil	100	0.13
	200	0.15

Contd.....

	400	0.20
	600	0.77
Clove oil	100	0.11
	200	0.12
	400	0.77
	600	0.93
Control	-	0.00

CD (p= 0.05); a =2.15; b= 2.74; axb=1.72
*Mean of three replications

observed in Streptomycin sulphate@ 600 ppm, followed by CS+BP (1.83cm) and Bronopol (1.83cm). Least (0.81cm) zone of inhibition was observed with CS at 100ppm concentration. The effectiveness of copper compounds against *Cmm* is reported [10, 16].

Among the *Trichoderma harzianum* isolates, PBAT-1 was found to be the best in which 89.5 per cent inhibition was recorded followed by B4 (87.8 per cent), B3 (84.5 per cent) and A1 (82.9 per cent). The bacterial bio-control agent PBAT-2 provided an effective control against the pathogen with a reduction of 91.3 percent in colony diameter when used alone, while in consortium with PBAT-1, it reduced the colony diameter upto74.5 per cent (Table 3). The isolates found effective were further screened under field conditions.

Table 3. Screening of biocontrol agents *C. michiganensis* subsp. *michiganensis* (Dual culture technique)

Treatment	Colony Diameter (mm)	Reduction in colony diameter (%)
PBAT-1	13.15	87.8
B1	35.15	61.3
B2	63.92	32.1
B3	17.21	84.5
B4	11.27	89.5
B5	44.10	54.5
B6	24.10	76.7
B7	57.47	32.2
A1	16.30	82.9
PBAT-2	8.13	91.3
PBAT-3	22.10	74.5

CD (p = 0.05) =1.36; SEm(±)= 0.46; CV= 2.82

Glasshouse Assays

Seeds of susceptible tomato variety Arka Vikas were inoculated with *Cmm* and further treated with different chemicals at 600ppm concentration found to be the most effective concentration in *in vitro* conditions. The perusal of the data (Table 4) indicates that maximum (72.34 per cent) per cent germination was in seeds treated with Streptocycline sulphate, followed by COC+BP (68.27 per cent).

Management of *Cmm* using varied copper compounds and streptomycin has been reported in previous studies. However, the increased dose of copper compounds was also observed to be phyto-toxic for the crop [8, 17, 18].

Amongst the biocontrol agents, the treatment showing highest seed germination was PBAT-1 (72.25 per cent) followed by PBAT-2 (67.24 per cent). Treatment with Root

Table 4. Screening of chemicals and biocontrol agents under glasshouse conditions through seed treatment

Treatments	Seed Germination (%)	Disease rating (Days after germination)					
		7	14	21	28	35	42
*Cmm*10	47.42	0	1	2	4	5	5
*Cmm*5	52.69	0	0	1	2	2	3
*Cmm*6	45.47	0	0	1	2	3	4
*Cmm*10+CS	55.62	0	0	1	2	2	3
*Cmm*10+COC	58.24	0	0	1	2	3	3
*Cmm*10+BP	65.30	0	0	1	1	2	3
*Cmm*10+SS	72.21	0	0	0	1	2	2
*Cmm*10+CS+BP	66.34	0	0	0	1	2	2
*Cmm*10+COC+BP	63.61	0	0	0	2	2	3
Cmm10+PBAT1	72.25	0	0	1	1	2	2
*Cmm*10+PBAT2	67.24	0	0	1	2	3	3
*Cmm*10+PBAT3	58.35	0	0	1	2	3	4
*Cmm*10+A1	69.27	0	0	1	1	2	2
Un inoculated Control	75.28	0	0	0	0	0	0

CD (p=0.05) = 1.86; CV = 1.79; SEm(±) = 0.64

Shield (*T. harzianum*) significantly reduced the incidence of bacterial canker on tomato plants and showed 63% reduction in disease. The study demonstrated that applications of 'Root Shield' (*T. harzianum*) applied as preventative foliar sprays were effective to control tomato bacterial canker caused by *C. michiganensis* subsp. *michiganensis* [19].

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