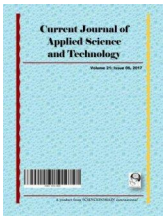

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Distribution and Variability of *Clavibacter michiganensis* subsp *michiganensis* in Tomatoes from Northern Hill Region of India

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

The objective of this research was to monitor the existence of *Clavibacter michiganensis* sub sp. *michiganensis* (*Cmm*) in tomato growing region in the northern hill region and to assess its cultural, morphological and pathogenic variability. Tomato samples showing symptoms of *Cmm* infection were collected and the causal agents were isolated from these samples. On the basis of the presence of characteristic symptoms of *Cmm* infection in tomato, the mean disease incidence and severity of suspected pathogen was found to be 19.57% and 16.11%, respectively in the middle Himalayan region. From a total of 80 tomato plants sampled, 17 bacterial isolates exhibiting similar colony morphology to *Cmm* were obtained. After the cultural, morphological and pathogenicity tests, 9 isolates were identified as *Cmm*. The results of this experiment can be a warning sign for all tomato growers in the region especially those involved in seed trade and vegetable export. For all the collected nine *Cmm* isolates studies on cultural and pathogenic variability were conducted under laboratory and greenhouse conditions. The design used for the experimental studies was completely randomised. Different strains of *Cmm* exhibited varied cultural and pathogenic variability while the morphological difference amongst the strains was observed to be non-significant, wherein all the ten

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isolates were of rod-shaped. During studies on cultural variability, most of the bacterial colonies were initially small, (1-4 mm diameter), mucoid and concave with three different pigmentations *viz.,* yellow, pale-white and orange which became glistening with the increase in age. Amongst the nine isolates studied the isolate, *Cmm* 10 was most virulent leading to complete plant death within 18th day of inoculation.

Keywords: C. michiganensis subsp michiganensis; tomato disease; field survey and pathogen variability.

1. INTRODUCTION

The tomato *(Lycopersicon esculentum* Mill.), belonging to family Solanaceae, is an economically important crop grown throughout the world and used as both fresh fruit and the processed food. It can be grown in a broad range of climatic conditions in the field and under protected cultivation [1]. It plays a major role in human nutrition and is an excellent source of phosphorus, iron and vitamin A, B and C [2,3,4]. The tomato growing and processing industry is of high significance, however, the cultivation of the tomato crop is heavily distressed by insect, fungi, bacteria and viruses which affect the fruit supply for its various industries. In the hilly region of northern India, the tomato crop is generally affected by different fungal, bacterial, viral and nematode diseases. Amongst bacterial diseases, the diseases that are often adding to the grower's losses are bacterial wilt (*Ralstonia solanacearum*) and bacterial spot (*Xanthomonas campestris* pv. *vesicatoria*) that have been reported earlier [5]. A bacterial disease of tomato caused by *Clavibacter michiganensis* subsp *michiganensis* having restricted spread in the country in Andhra Pradesh, Karnataka, Madhya Pradesh, Maharashtra and Tamilnadu [6] is being observed in the northern hill region since past few years. An extreme reduction in the qualitative and quantitative parameters is being done by the bacterium, leading to significant monetary loss both in protected and open field cropping conditions; which sometimes may even lead to complete yield loss [7,8,9]. The pathogen being seed-borne in nature, has been categorised by European Plant Protection Organisation (EPPO) as an A2 category quarantine phytopathogen, occurring in almost all the tomato growing areas round the globe [10]. Contaminated seeds act as the source of long distance dispersal of bacterial inoculum [11, 12]. However, bacterial canker of tomato has not been yet studied and documented from the tomato growing areas of outer himalayan belt. Keeping in view the importance of the crop and heavy losses caused by this disease, the specific objectives of this research were (i) Collection of tomato fruits and plants samples of showing characteristic symptoms of *Cmm,* (ii) to estimate disease incidences and severity in the field based on disease symptoms (iii) to identify the presence of bacterium in the infected tomato samples, and (iv) to determine the distribution of *Cmm* in various tomato growing region in northern hills.

2. MATERIALS AND METHODS

2.1 Disease Survey and Sample Collection

A detailed survey was carried out for recording the occurrence and spread of bacterial canker in tomato growing regions of a middle of Himalayas (Two districts of Himachal Pradesh *viz.,* Solan and Sirmaur) during cropping seasons of 2015-16 (Table 1). The incidence and severity of bacterial canker were observed at different locations in the field. Around three tomato fields per location were visited and disease incidence was calculated with the help of following formula:

Disease incidence (%) = (Number of diseased plants/ Total number of plants observed) × 100

The per cent disease severity was calculated as per the method given by Kawaguchi et al. [13]

Disease severity (%) = (Sum of all disease ratings/ Total number of ratings × Maximum disease grade) × 100

2.2 Isolation, Purification and Preservation of the Bacterium

The bacterium was isolated, from infected seeds, seedlings, plant parts and fruits, purified and preserved [11] for subsequent studies. Infected tissues were surface sterilised by alcohol (70%) and were then placed in a test tube containing sterilised water. Tissue from each infected plant

part was left for 30 minutes in the sterile water and crushed gently so that the bacterium can be released out of the tissue into the water. Subsequently, 100 µl of suspension was poured into petriplate containing Nutrient Agar Glucose Yeast medium (NGY). The seeds collected from infected fruits exhibiting the disease symptom were placed on the NGY medium and incubated in the growth chamber at 28±1ºC. The seeds were examined after 72 h to record the recovery of the bacterium.

Table 1. List of *Cmm* isolates used in the present study

S. No.	Isolate	Designation
1	Nauni	*Cmm* 1
2	Khaltu	*Cmm* 2
3	Kalaghat	*Cmm* 3
4	Kotla Panjola	*Cmm* 4
5	Garganoo	*Cmm* 6
6	Narag	*Cmm* 7
7	Kyar	*Cmm* 8
8	Nainatikkar	*Cmm* 9
9	Deothal	*Cmm* 10

Purification of the bacterium was done on D_2ANX medium, a semeiselective medium for *Cmm* isolation [12] that shows the characteristic feature of the bacterium. The freshly growing single colonies (48 h) from NGY medium were streaked into D_2ANX medium and incubated at 28 ± 1ºC for 5 days. *Cmm* strains were stored in NGY slants at 4ºC and in glycerol at -20ºC for further studies.

2.3 Studies on Variability of *Clavibacter michiganensis* subsp *michiganensis* Isolates

2.3.1 Cultural variability of *Cmm* isolates

The cultural characteristics of the bacterium were observed on 3rd day after incubation for all the isolates of *Cmm.* Characteristics like colony pigmentation (colour), shape and size were recorded by direct observation of the culture grown in petri-plates containing NGY medium. For this purpose nine selected bacterial isolates of *Cmm* were taken (*Cmm*1, 2, 3, 4, 6, 7, 8, 9 and 10) and were tested for their cultural variation on NGY medium. For each isolates five plates were poured with the medium. After solidification, each of the bacterial isolates was streaked into the medium and was incubated at 28±1ºC for 72 h. After 72 h of incubation, the plates were examined for bacterial growth and colony morphology.

2.3.2 Pathogenic variability of *Cmm* isolates

All the nine isolates of *Cmm* (*Cmm*1, 2, 3, 4, 6, 7, 8, 9 and 10) were evaluated for the expression of wilt and canker symptom under glass house conditions on the tomato variety Arka Vikash. For that purpose, seeds of tomato variety "Arka Vikash" were sown in pots (15 x 10 cm in dia.) filled with sterilised soil + sand (2: 1: w/w) and kept at alternating 14 h :10 h alternating light : dark period and at 30º ± 2ºC . One seedling was maintained per pot. The plants were inoculated with the pathogen at 5th week stage.

For the preparation of bacterial cell suspension, one loop full culture of 48 h old bacterial colony was inoculated into 50 mL of autoclaved nutrient broth in 150 ml "Erlenmayer flask. The suspension was adjusted to an OD value of 0.06 at 660 nm that corresponds to 10^8 cfu ml^{-1} by adding required quantity of sterilised distilled water. Pathogenicity tests were conducted on five week old tomato seedlings.

The seedlings were inoculated with a sterilised hypodermic syringe in the stem region of five week old seedlings with 0.2 ml of standardised bacterial suspension (1.0 x 10^8 cfu ml^{-1}). Plants were kept under alternate 14h light and 10h dark periods at 30º ± 2ºC temperature and were observed periodically for the appearance of the symptoms.

Table 2. Disease rating scale for *Cmm*

Rating scale	Disease score	Symptoms
0	No disease	no leaves showing wilting
1	1-/10% of leaves with wilt	slight marginal wilting,
2	11-/25% of leaves with wilt	Unilateral wilting of the leaves
3	26-/49% of leaves showing wilting	sectored wilting, and canker formation, associated with chlorosis
4	50-/74% of leaves showing wilting	pronounced leaf collapse
5	whole leaf wilted	Complete plant death

2.3.3 Observations

Data on disease development was recorded on the basis of percent wilting and canker formation on the stem. Evaluation of disease appearance and development was determined using 0-/5 arbitrary scale [14].

2.3.4 Studies on growth of bacterium in different temperature and pH conditions

To know the effect of temperature and pH on multiplication of the bacterium, the bacterial strains were incubated at six different temperature (10ºC, 15ºC, 20ºC, 25ºC, 30ºC, 35ºC, 45ºC) and five different pH (4, 5, 6, 7 and 8). For evaluating the effect of temperature, 150 ml 'Erlenmeyer' flasks, each containing 50 ml of sterilised NGY broth (pH 7.0) were autoclaved at 15 lb / in^2 for 20 minutes to avoid contamination of the media. The sterilised broth was inoculated with 48h culture using straight inoculation and then incubated at a series of temperature ranging from 10ºC, 20ºC, 30ºC and 40ºC. The flasks containing the inoculated broth were then incubated at the specified temperature and observations were recorded at 72 h after incubation. The OD value at 660 nm by using spectrophotometer was observed for each replication for each temperature. For evaluating the effect of pH on the growth of the bacterium 150 ml 'Erlenmeyer' flasks, each containing 50 ml of NGY broth were maintained at pH 4, 5, 6, 7 and 8 followed by autoclaving and inoculation of the bacterial suspension. The flaks were then incubated at 28 ± 1ºC and observations were recorded at 72 h after incubation. For 72 h observing the effect of temperature and at pH range of 4, 5, 6, 7, and 8. Each treatment was replicated thrice.

2.4 Statistical Analysis

The data was analysed using simple ANNOVA on Completely Randomized Design (CRD) in *invitro* studies and Randomized Block Design (RBD) in *invivo* studies. 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.

3. RESULTS AND DISCUSSION

3.1 Symptomatology

The pathogen *Clavibacter michiganensis* subsp *michiganesis* (Smith) Davis showed an array of symptoms in tomato seedlings and plants. Artificial infection through inoculation of the pathogen on different plant parts exhibited marginal necrosis in the leaves, canker on stem and fruits and unilateral wilting followed by wilting of the entire plant leading to the plant death.

Under natural field conditions, the disease is generally observed during the months of May-August, where high temperature is accompanied by rainfall. The rain splashes act as the resource for the spread of the bacterium. The symptoms of the disease became visible on the stem region as cankerous lesion, which increases in size chronically (A), infection on the stem often also girdles the stem and may cause premature plant death, The symptom on the leaves appears in the form of unilateral wilting in plant (B), that appeared starting from the top most part and follows downside, whole plant wilting (C), symptom on the fruit appeared in the form of bird´s eye spot formation (D) (Plate 1). The pathogen produces an array of symptom varying on the basis of plant stages, time of infection, intercultural operations, location of production under glasshouse or field conditions and cultivar etc [15].

3.2 Disease Incidence and Severity of *Cmm*

The magnitude of bacterial canker of tomato was assessed through systematic surveys of tomato growing areas of Himachal Pradesh conducted during the crop season of 2015-16 and 2016-17 at vegetative and fruiting stage of the crop growth. The data on disease incidence and disease severity were recorded and presented in Table 3.

Table 3. Disease incidence and severity of bacterial canker in tomato growing areas of Himachal Pradesh

Location	Disease incidence (%)	Disease severity (%)
Nauni	27.88	23.64
Khaltu	32.69	28.48
Kalaghat	18.01	12.31
Kotla Panjola	16.24	14.12
Deothal	64.23	58.39
Garganoo	36.22	32.35
Narag	31.82	26.70
Kyar	38.94	31.22
Nainatikkar	26.36	22.26
Mean	33.59	27.71
Overall mean	19.57	16.11

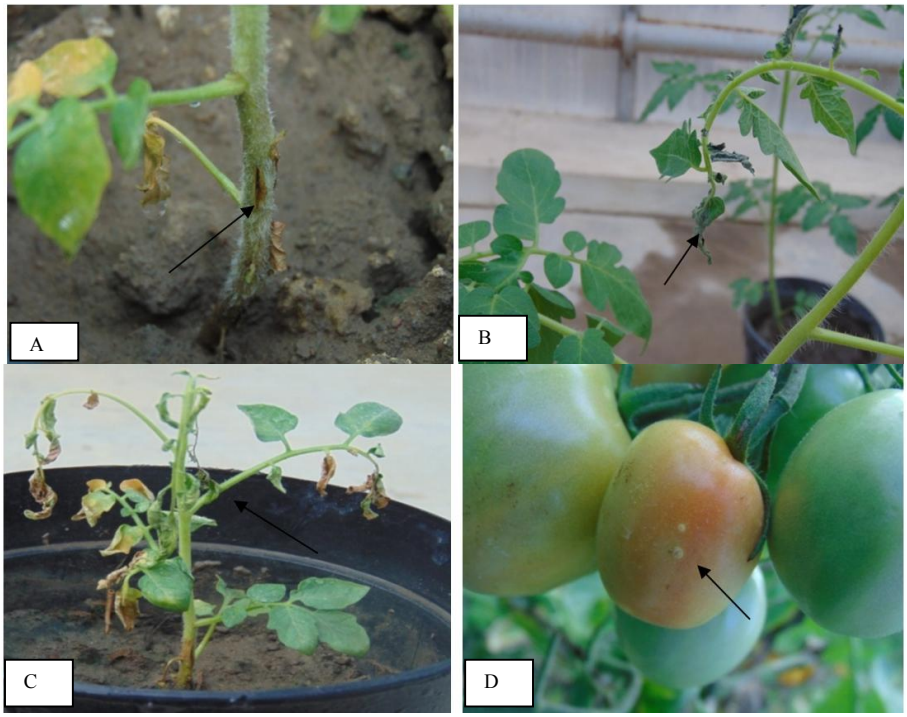

Plate 1. Symptom expression by *Cmm* on different parts of tomato plant; A, showing canker on stem of the tomato seedling; B. Unilateral wilting of the leaves of tomato seedlings; C. whole plant wilting; D canker on tomato fruits

It is apparent from the data in Table 3 that the overall incidence and severity of bacterial canker of tomato was observed to be 19.57% and 16.11 %, respectively. During the survey of Himachal Pradesh the disease was found to be widely spread in most of the tomato growing region of the state with a varying percentage of mean disease incidence and severity. In HP, the disease incidence and diseases severity was found to be 33.59% and 27.71%, respectively. The maximum disease incidence and severity was 64.23% and 58.39%, respectively at Deothal region followed in Kyar where the diseases incidence and severity has been 38.94% and 31.22%, and in Gargano region of Himanchal Pradesh (HP) where the disease incidence was 36.22% and 32.35%, respectively. The lowest disease incidence of 16.24% and severity of 14.12% was observed at Kotla Panjola in HP. The occurrence of bacterial canker in tomato fields in the state Karnataka with an average incidence of 48% have been reported [16,17] and the prevalence of the disease in Himachal Pradesh has also been reported [18]. The nature of *Cmm* is described to be seed-borne that plays an important role in the long distance dissemination of the pathogen [19]. The pathogen from this region has not been reported by EPPO in its last documentation [6] which indicates that *Cmm* may have been transported in the tomato growing areas of Himachal Pradesh through contaminated seed. The seed borne nature of the pathogen makes the use of certified *Cmm* free seed essential [7,20]. Even though less than 1% of *Cmm* transmission has been reported through infected seed but 0.01 to 0.05% of infested seed are sufficient to cause an epidemic in suitable environmental conditions for multiplication and dissemination of the bacterium [7].

3.3 Studies on Variability of *Clavibacter michiganensis* subsp *michiganensis* Isolates

3.3.1 Cultural variability of different isolates of *Cmm*

On NGY medium, the bacterial colonies of most of the isolates were small, 1-4 mm in diameter and developed within 72-96 h from the day of inoculation. The bacterial colonies were light yellow, orange, round and semifluidal (Plate 2).

Colonies become deeper yellow and glistening with longer period of incubation (Table 4). The colony colour of the bacterial isolates *Cmm*1, 3, 4, 6 and 8 was yellow and that of isolate *Cmm*2 was creamish white to yellow while it was orange in isolate *Cmm*5, 7, 9 and 10.The shape of colonies was concave to dome-shaped in case of all the isolates. Different colony characteristics including dry, sticky, mucoid and less mucoid, pink, red, yellow, orange, white or colourless colonies are also been reported [21,22].

3.3.2 Pathogenic variability of different isolates of *Cmm*

Nine bacterial isolates were inoculated into the variety "Arka Vikash" by syringe inoculation method at the nodal point of true leaf emergence and the observation for disease rating was taken.

Artificial inoculation of the tomato seedlings with different isolates revealed that the seedlings inoculated with isolates *Cmm* 6 and *Cmm* 10 showed first symptom on leaf as marginal necrosis and unilateral wilting on 6th day of inoculation (Table 5). Isolates, *Cmm*1, *Cmm* 2, *Cmm* 3, *Cmm* 8 and *Cmm* 9 showed symptoms on leaf on 12th day of inoculation, while isolates *Cmm* 4 and *Cmm* 7 showed symptoms on 18th day of inoculation. Isolate *Cmm* 10 was the most virulent which caused complete plant death within 18th day of inoculation, while isolate *Cmm* 8 was observed to be the least virulent amongst the all 10 isolates and showed significant symptoms on 5th week after inoculation. Isolate *Cmm* 10 which show the signs of the most virulent isolate was further taken up for glasshouse and open field studies. The scoring for disease severity was started since the period of inoculation up to a period of 7 weeks. The isolates were characterised further on the basis of their virulence character (Table 6). Variation in the virulence of *Cmm* strain was also detected [23] in New York and Silicy [24].

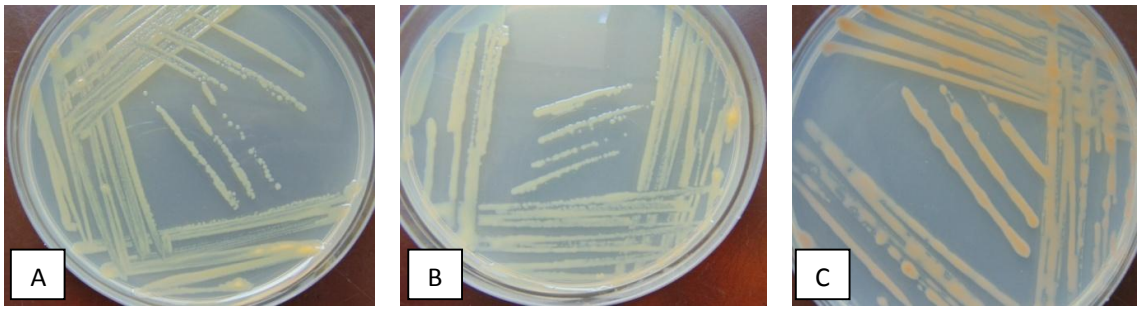

Plate 2. Cultural variability of different isolates (*Cmm*1-*Cmm*10) of the bacterium (A-I).
A= *Colony pigmentation of isolates Cmm 1, 3, 4*
B= Colony pigmentation of isolates Cmm 2
C= Colony pigmentation of isolates Cmm 6, 7, 8, 9, 10

Table 4. Colony characteristics of different isolates of bacterial canker pathogen on nutrient agar glucose yeast medium

S. No.	Isolates	Colony characteristics		
		Colour	Shape	Size (mm)
A	*Cmm1*	Yellow	Round, mucoid	1-2
B	*Cmm2*	Creamish white to yellow	Round	1-3
C	*Cmm3*	Yellow	Circular, fluidal	1-3
D	*Cmm4*	Yellow	Circular	2-4
E	*Cmm6*	Orange	Round	1-3
F	*Cmm7*	Orange	Round	1-3
G	*Cmm8*	Orange	Circular, fluidal	2-4
H	*Cmm9*	Orange	Circular	2-3
I	*Cmm10*	Orange	Round, mucoid	1-3

Plate 3. Pathogenic variability of different isolates of the pathogen on variety Arka Vikash (*Cmm*1- *Cmm10*)

Table 5. Pathogenic variability of *Cmm* isolates on tomato seedlings*

Isolates	Method of inoculation	Days after inoculation							
		0	6	12	18	24	30	36	42
Cmm1	Stem inoculation	0	0	1	2	3	3	3	4
Cmm2	Stem inoculation	0	0	1	2	3	4	4	4
Cmm3	Stem inoculation	0	0	1	2	3	3	4	4
Cmm4	Stem inoculation	0	0	0	1	2	3	3	3
Cmm6	Stem inoculation	0	1	2	3	4	4	4	4
Cmm7	Stem inoculation	0	0	0	1	2	2	3	3
Cmm8	Stem inoculation	0	0	1	1	2	2	2	2
Cmm9	Stem inoculation	0	0	1	1	2	3	3	3
Cmm10	Stem inoculation	0	3	4	5	5	5	5	5

** Results based on five scale rating as detailed in Table 1*

Table 6. *Cmm* isolates categorisation on the basis of virulence

Isolate	Virulence categorisation
Cmm 10	Highly virulent
Cmm 1,2,3,4,6,7,9	Virulent
Cmm 8	Hypo virulent

3.3.3 Studies on growth of bacterium in different temperature conditions

The optimum temperature for the growth of *Cmm* was assessed through growing the nine virulent bacterial isolates *Cmm* (1, 2, 3, 4, 6, 7, 8, 9 and 10) in the following range of temperature.

The studies for assessing the most suitable temperature for the growth and multiplication of the nine virulent isolates on a range of temperatures *viz.,* 10ºC, 15ºC, 20º, 25ºC, 30ºC, 35ºC and 45ºC was done, wherein, 25ºC temperature was found to be the optimum temperature for the growth and multiplication of all the isolates (Table 7). The growth of *Cmm* in the similar temperature ranges has been also reported earlier [22] which confirm 25ºC - 30ºC as optimum temperature range for pathogen growth.

3.3.4 Studies on growth of bacterium at different pH conditions

To determine the optimum pH for the growth of *Cmm* the nine virulent bacterial isolates *Cmm* (1, 2, 3, 4, 6, 7, 8, 9 and 10) were subjected to the pH range of 4-8.

Amongst the varied pH conditions observed for the growth of the bacterium pH 7 was found to be the optimum temperature for bacterial growth (Table 8). The results are in agreement with the findings of the earlier studies [18], where the researcher confirmed the pH range 6 - 7 optimal for bacterial growth.

Table 7. Growth of bacterium in different temperature conditions

Isolates	OD value (660nm) of bacterial isolates at different temperature						
	10ºC	15ºC	20ºC	25ºC	30ºC	35ºC	45ºC
Cmm1	0.480	0.946	1.163	1.253	1.280	0.956	0.153
Cmm2	0.327	0.528	0.892	1.182	1.205	0.813	0.236
Cmm3	0.946	1.053	1.263	1.336	1.263	1.056	0.190
Cmm4	0.209	0.492	0.813	1.201	1.225	0.926	0.343
Cmm6	0.950	1.146	1.263	1.350	1.256	1.153	0.273
Cmm7	0.416	0.917	1.256	1.319	1.284	0.918	0.307
Cmm8	0.450	0.950	1.070	1.176	1.130	0.970	0.260
Cmm9	0.315	0.502	0.935	1.215	1.275	1.192	0.115
Cmm10	0.946	1.160	1.271	1.353	1.256	0.940	0.160
CD at 5%	a= 0.0155; b= 0.0168; axb=0.0412						

a= interaction within the isolates
b= interaction within the temperature conditions
axb= interaction between isolates and temperature conditions

Table 8. Growth of bacterium in different pH conditions

Isolates	OD value (660nm) of bacterial isolates at different pH				
	4	5	6	7	8
*Cmm*1	0.070	0.80	1.34	1.43	1.26
*Cmm*2	0.031	0.094	0.62	1.18	1.05
*Cmm*3	0.046	0.14	0.81	1.26	0.95
*Cmm*4	0.061	0.11	0.72	1.25	1.13
*Cmm*6	0.070	0.36	1.28	1.35	1.16
*Cmm*7	0.038	0.89	1.19	1.23	1.10
*Cmm*8	0.040	0.25	1.24	1.32	1.15
Cmm9	0.018	0.12	0.83	1.07	0.92
*Cmm*10	0.073	0.45	1.36	1.47	1.24
CD at 5%	a = 0.11;	b = 0.101; axb = 0.247			

a= interaction within the isolates
b= interaction within the pH conditions
axb= interaction between isolates and pH conditions

4. CONCLUSION

The information presented here highlights the spread of *Cmm* into the Northern region of India, where it was not been observed earlier. Thereby indicating the existence and spread of the pathogen into the newer tomato growing region of the country as well as the presence of pathogenic and cultural variability amongst the *Cmm* strains, exhibited by the differential infection rate and varied colony characteristics respectively. Hence, it is essential for the tomato growers to use only Cmm free certified seeds, to restrict the entry of the pathogen into new areas. This would certainly lead to a reduction in the disease incidence and also in the economic loss caused to the crop growers by the pathogen.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Atherton J, Rudich J. (Eds.). The tomato crop: A scientific basis for improvement. Springer Netherlands. 1986;661.
2. Cobley LS, Steele WM. An introduction to the botany of tropical crops. Second edition. Longman Group. Limited London; 1976.
3. Varela AM, Seif A, Lohr B. A guide to IPM in tomato production in Eastern and Southern Africa. CTA/ ICIPE/ GTZ; 2003.
4. Naika S, van Lidt de Jeude J, de Goffau M, Hilmi M, van Dam B. Cultivation of tomato: Production, processing and marketing. CTA. 2005;(Series No 17).
5. Gupta SK, Thind TS. Disease problems in vegetable production. Scientific Publishers (India), Jodhpur. 2006;576.
6. EPPO/CABI. Invasive species compendium. Wallingford, UK: CAB International; 2014.
   Available:www.cabi.org/isc
7. Chang RJ, Ries SM, Pataky JK. Local sources of *Clavibacter michiganensis* ssp. *michiganensis* in the development of bacterial canker on tomatoes. Phytopathology. 1992;82(5):553-560.
8. Gitaitis RD, Beaver RW, Voloudakis AE. Detection of *Clavibacter michiganensis subsp. michiganensis* in symptomless tomato transplants. Plant Dis. 1991;75: 834–838.
9. Hadas R, Kritzman G, Klietman F, Gefen T, Manulis S. Comparison of extraction procedures and determination of the detection threshold for *Clavibacter michiganensis* ssp. *michiganensis* in tomato seeds. Plant Pathol. 2005;54(5): 643-649.
10. EPPO/CABI. Map 253 in: Distribution maps of quarantine pests for Europe. CAB International, Wallingford, GB; 1998.
11. De Leon L, Siverio F, Lopez M, Rodriguez A. Comparative efficiency of chemical compounds for *in vitro* and *in vivo* activity against *Clavibacter michiganensis subsp michiganensis*, the causal agent of tomato bacterial canker. Crop Prot. 2008;27(9): 1277-1283.
12. Chun WCC. Identification and detection of *Corynebacterium michiganense* in tomato seed using the indirect enzyme-linked immunosorbent assay. MSc Thesis.

Honolulu, HI, USA: University of Hawaii; 1982.
13. Kawaguchi A, Tanina K, Inoue K. Molecular typing and spread of *Clavibacter michiganensis* subsp. *michiganensis* in greenhouses in Japan. Plant Patho. 2010; 59(1):76–83.
14. Soylu S, Baysal O, Soylu EM. Induction of disease resistance by the plant activator, acibenzolar-S-methyl (ASM), against bacterial canker (*Clavibacter michiganensis* sub sp. *michiganensis*) in tomato seedlings. J. Plant Pathol. 2013;85(3):175-181
15. EPPO bulletin. PM 7/42 (3). *Clavibacter michiganensis* subsp. *michiganensis*. OEPP/EPPO Bulletin. 2016;46:202–225.
16. Sarala IS, Shetty S. *Clavibacter michiganensis* subsp. *michiganensis* in tomato fields of Karnataka, India. Adv. Plant Sc. 2005;18(2):581-589.
17. Umesha S. Occurrence of bacterial canker in tomato fields of Karnataka and effect of biological seed treatment on disease incidence. Crop Prot. 2006;25:375–381.
18. Singh G, Bharat NK. Studies on bacterial canker (*Clavibacter michiganensis* subsp. *michiganensis*) of tomato (*Solanum lycopersicum*). Int J Curr Microbiol Appl Sci. 2017;6(9):317-323.
19. Tsiantos J. Transmission of bacterium *Corynebacterium michiganense* pv. michiganense by seeds. J. Phytopathol. 1987;119:142-146.
20. Mansfeld-Giese K. Plant-to-plant transmission of the bacterial ring rots pathogen *Clavibacter michiganensis* subsp. sepedonicus. Potato Res. 1997;40:229–235.
21. Davis MJ, Vidaver AK. Gram positive bacteria coryneform plant pathogens. In: Schaad NW, Jones JB, Chun W. eds. Laboratory guide for identification of plant pathogenic bacteria, 3rd edn. St. Paul, MN, USA: APS Press. 2001;218–235.
22. Kaneshiro WS, Alvarez AM. Specificity of PCR and ELISA assays for hypovirulent and avirulent *Clavibacter michiganensis* subsp. *michiganensis*. Phytopathology. 2001;91:S46.
23. Tancos MA, Lange HW, Smart CD. Characterizing the genetic diversity of the *Clavibacter michiganensis* subsp. *michiganensis* population in New York. Phytopathology. 2015;105(2):169–179.
24. Ialacci GM, Bella P, Licciardello G, Strano C, Eichenlaub R, Gartemann KH, et al. Clonal populations of *Clavibacter michiganensis* subsp. *michiganensis* are responsible for the outbreaks of bacterial canker in greenhouse tomatoes in Italy. Plant Path. 2015;65(3):484–495.

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