



# Cultural Parameters of *Xanthomonas cucurbitae* Causing Bacterial Leaf Spot of Bottle Gourd and Pumpkin

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## Abstract

Bacterial leaf spot caused by *Xanthomonas cucurbitae* is an important disease of cucurbits leading to huge crop losses especially to bottle gourd, pumpkin and squashes in sub tropical zone of Himachal Pradesh. Cultural parameters like effect of different temperatures, pH levels and nutrient media on the growth of two isolates of *X. cucurbitae* isolated from bottle gourd and pumpkin were studied under *in vitro* conditions. Among various temperature regimes (15–35 °C) tested for both the isolates, a temperature range of 25 to 30 °C was observed to be optimum, for bottle gourd ( $5.35 \times 10^7$  cfu ml<sup>-1</sup> at 25 °C) and pumpkin ( $4.15 \times 10^7$  cfu ml<sup>-1</sup> at 30 °C) isolates. Out of six pH levels (4.0–9.0) tested to see the effect on bacterial growth, the optimum pH range for maximum growth of the bacteria was observed to be 6.0 to 7.0 for bottle gourd ( $44.16 \times 10^7$  cfu ml<sup>-1</sup> at pH 6.0) and pumpkin ( $31.62 \times 10^7$  cfu ml<sup>-1</sup> at pH 7.0) isolates. However, no growth of the isolates was recorded at pH 4.0. The bacterium grew best in yeast extract calcium carbonate broth ( $8.11 \times 10^7$  cfu ml<sup>-1</sup> and  $10.63 \times 10^7$  cfu ml<sup>-1</sup>; bottle gourd and pumpkin isolates, respectively), nutrient glucose broth ( $9.31 \times 10^7$  cfu ml<sup>-1</sup>; bottle gourd isolate) and nutrient sodium chloride broth ( $7.70 \times 10^7$  cfu ml<sup>-1</sup> and  $7.33 \times 10^7$  cfu ml<sup>-1</sup>; bottle gourd and pumpkin isolates, respectively).

**Keywords:** *Xanthomonas cucurbitae*, cultural characteristics, bacterial spot, cucurbits

## 1. Introduction

*Xanthomonas cucurbitae* (Bryan) Vauterin et al. (Syn. *X. campestris* pv. *cucurbitae*) causing bacterial spot is emerging as an important pathogen leading to huge crop losses especially to bottle gourd, pumpkin and squashes (Jarial et al., 2011; Babadoost, 2012; Babadoost, 2017). This disease was first reported as bacterial leaf spot on Hubbard squash in New York in 1925 by Bryan (Bryan, 1930). Since then, the disease has been reported to occur on various cucurbits (Gorlenko, 1979; Vlasov, 2005) like squash (Robbs et al., 1972; Alippi, 1989; Kushima et al., 1994; Banhero et al., 1998; Liu et al., 2016), cucumber (Vincent-Sealy and Brathwaite, 1982; Maringoni et al., 1988; Sinha, 1989; Lia et al., 2018), pumpkin (Pruvost et al., 2009; Lamichhane et al., 2010; Babadoost and Ravanlou, 2012; Salamanca, 2014; Trueman et al., 2014; Ravanlou and Babadoost, 2015), watermelon (Pruvost et al., 2009; Dutta et al., 2013)

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and on bottle gourd (Jarial et al., 2011; Jarial et al., 2015; Sharma, 2016) from different countries of world. The disease has been reported to cause significant losses in different cucurbits. According to Larazev (2009), yield losses reach more than 20% in susceptible cultivars however, the disease severity reaches up to 50-60% at fruit storage in various cucurbits. Jarial et al. (2011) reported that yield losses in bottle gourd may reach up to 20 to 70%. However, in case of pumpkin, up to 90% losses have been reported by Salamanca (2014).

Bryan (1930) described the cultural characters of the bacterium for the first time. According to him, *X. campestris* pv *cucurbitae* is a short, rod shaped bacterium, 0.5 to 1.3×0.45 to 0.6  $\mu$  in diameter with one polar flagellum occurring singly in pairs or in short chains. It is a Gram-negative, non acid-fast and non-spore producing bacterium. On beef agar, the colonies are round, yellow and opalescent. In general, Schaad and Stall (1988) have suggested a temperature range of 25 to 27 °C to be conducive for the growth of all the species of *Xanthomonas*. Many reports are not available in the literature regarding the cultural studies of *X. cucurbitae*. However, Zhang and Babadoost (2018) reported that the optimum temperature for colony development ranged from 24 °C to 30 °C and the maximum colony growth was observed at pH 6.5 to 7.0. Therefore, understanding of the nature of the pathogen with respect to cultural and physiological features is felt necessary and present studies were conducted with an objective to study the effect of different temperature regimes, pH levels and nutrient media on the cultural growth of *X. cucurbitae*.

## 2. Materials and Methods

All experiments on cultural studies were conducted in the laboratory, Department of Plant Pathology, College of Horticulture and Forestry, Neri, Hamirpur, Himachal Pradesh. The treatments were designed in completely randomized block design with three replications. The statistical analysis was done by using online software OPSTAT.

### 2.1. Isolation, purification and maintenance of the pathogen

Infected leaves of bottle gourd and pumpkin exhibiting characteristic symptoms were brought to laboratory for isolation of the pathogen. The infected portions of the leaves were swabbed with rectified spirit followed by 1% sodium hypochlorite and washed repeatedly in sterile distilled water. Small pieces of diseased tissues were cut from the affected leaves with the help of sterilized blade. The bits were placed in sterile water drops in a sterilized Petri plate under aseptic conditions. In order to obtain the bacterial ooze, each bit was incised repeatedly with a sterile blade. Simultaneously, one drop of sterile water containing bacterial ooze was also examined under the microscope for the presence of bacterial cells. A loopful of bacterial suspension was streaked on sterilized nutrient sodium chloride agar (NSA) plates under aseptic conditions. These Petri plates were incubated at 30 °C for 72 h and observed for colony formation of the pathogen.

Typical colonies characteristic of the bacterium were picked

from the Petri plates and transferred to Petri plates containing nutrient sodium chloride agar medium by streak plate method for the purification of culture. These cultures were further purified by streak plate method on NSA slants and maintained at 4-5 °C in refrigerator for further studies. The culture was periodically sub-cultured at an interval of two weeks regularly.

### 2.2. Effect of different temperature regimes on the growth of *X. cucurbitae*

Erlenmeyer flasks of 150 ml capacity each containing 50 ml of nutrient sodium chloride broth were sterilized and inoculated with 1 ml of bacterial suspension (48 h old culture of both isolates in nutrient broth) and incubated at different temperatures viz., 15, 20, 25, 30 and 35 °C for 48 h. Data were recorded in terms of colony forming units per millilitre (cfu ml<sup>-1</sup>) depicting bacterial growth. For this, the bacterial suspension thus obtained after incubation in each flask was diluted serially up to 10<sup>-7</sup> and pour plated on nutrient sodium chloride agar medium with 1 ml bacterial suspension and incubated at 30°C for 72 h to record the colony forming units ml<sup>-1</sup> (cfu ml<sup>-1</sup>).

### 2.3. Effect of different pH levels on the growth of *X. cucurbitae*

The effect of different pH levels viz., 4.0, 5.0, 6.0, 7.0, 8.0 and 9.0 on the growth of the bacterium (both isolates i.e. bottle gourd and pumpkin) was evaluated so as to find the optimum pH for its growth. The desired pH levels were adjusted with the help of 1N HCl or NaOH. For this, 'Erlenmeyer flasks' (150 ml capacity) containing 50 ml nutrient sodium chloride broth adjusted with different pH levels were inoculated with 1 ml bacterial suspension (48 h old culture of both isolates in nutrient broth) and incubated at the best temperature obtained in previous experiment for 48 h. Data were recorded in terms of cfu ml<sup>-1</sup> as mentioned under previous experiment.

### 2.4. Effect of different liquid media on the growth of *X. cucurbitae*

Different nutrient liquid viz., nutrient sodium chloride broth, nutrient glucose broth, yeast extract nutrient broth, yeast extract sucrose peptone broth, yeast extract dextrose calcium carbonate broth and nutrient yeast extract broth were evaluated *in vitro* by colony count to study their effect on the growth of both isolates of *X. cucurbitae*. For this, 50 ml broth of each medium was taken in 150 ml capacity Erlenmeyer flasks and autoclaved at 15 lbs psi for 20 minutes and inoculated with 1 ml bacterial suspension taken from 48 h old culture in nutrient broth. After 72 h of incubation, the bacterial suspension thus obtained in each nutrient medium was serially diluted and pour plated as mentioned above to record the cfu ml<sup>-1</sup>.

## 3. Results and Discussion

The colony characteristics of both pathogen isolates were observed on NSA medium. The colonies were mucoid, circular, smooth textured and yellow in colour having diameter of



about 2-4 mm.

3.1. Effect of different temperature regimes on the growth of *X. cucurbitae*

Among various temperature regimes ranging from 15-35 °C tested for both isolates (Table 1), maximum growth ( $5.35 \times 10^7$  cfu ml<sup>-1</sup>) of bottle gourd isolate was recorded at 25 °C which was significantly higher than the remaining temperature treatments followed by growth at 30 °C ( $3.80 \times 10^7$  cfu ml<sup>-1</sup>). The growth of bottle gourd isolate at temperatures 20 °C ( $2.31 \times 10^7$  cfu ml<sup>-1</sup>), 15 °C ( $2.18 \times 10^7$  cfu/ml) and 35 °C ( $1.63 \times 10^7$  cfu ml<sup>-1</sup>) was found to be statistically at par with each other.

Table 1: Effect of different temperature regimes on the growth of *Xanthomonas cucurbitae*

Temperature (°C)	Growth of bacterial isolates (×10 <sup>7</sup> cfu ml <sup>-1</sup> )	
	Bottle gourd	Pumpkin
15	2.18 <sup>c</sup>	2.15 <sup>c</sup>
20	2.31 <sup>c</sup>	3.71 <sup>b</sup>
25	5.35 <sup>a</sup>	4.15 <sup>b</sup>
30	3.80 <sup>b</sup>	4.68 <sup>a</sup>
35	1.63 <sup>c</sup>	2.25 <sup>c</sup>
CD (p=0.05)	0.85	0.51
SEm±	0.26	0.16

In case of pumpkin isolate, data indicate that significantly maximum growth of the bacterium was recorded at 30 °C ( $4.68 \times 10^7$  cfu ml<sup>-1</sup>) followed by growth at 25 °C ( $4.15 \times 10^7$  cfu/ml) which was statistically at par with the growth at 20 °C ( $3.71 \times 10^7$  cfu ml<sup>-1</sup>). Minimum growth of the bacterium was recorded at 15 °C ( $2.15 \times 10^7$  cfu ml<sup>-1</sup>) which was statistically at par with the growth recorded at 35 °C ( $2.25 \times 10^7$  cfu ml<sup>-1</sup>). Thus, temperatures of 25 and 30 °C were found to be optimum for the growth of bottle gourd and pumpkin isolates, respectively.

The results of temperature studies of *X. cucurbitae* obtained in present investigations were in accordance with Babadoost and Zitter (2009) and Zhang and Babadoost (2018) who reported that optimum temperature for the growth of *X. cucurbitae* ranged from 24 to 30 °C. The present investigations are further supported by Maji and Nath (2015) and Suresh et al. (2013) who reported that 30 °C is optimum temperature for the growth of *Xanthomonas* species.

3.2. Effect of different pH levels on the growth of *X. cucurbitae*

The pathogen grew to a variable extent at all pH levels tested except pH 4.0 at which no growth of the pathogen was observed. In case of bottle gourd isolate, maximum growth ( $44.16 \times 10^7$  cfu ml<sup>-1</sup>) of the pathogen was recorded at pH 6.0 which was significantly higher than the growth at remaining pH levels tested followed by growth at pH 7.0 ( $36.15 \times 10^7$  cfu ml<sup>-1</sup>), 5.0 ( $25.33 \times 10^7$  cfu ml<sup>-1</sup>), 8.0 ( $6.80 \times 10^7$  cfu ml<sup>-1</sup>) and 9.0

( $4.16 \times 10^7$  cfu ml<sup>-1</sup>).

In case of pumpkin isolate, significantly maximum growth ( $31.62 \times 10^7$  cfu ml<sup>-1</sup>) of the pathogen was recorded at pH level 7.0 followed by pH 6.0 ( $27.03 \times 10^7$  cfu/ml) and pH 5.0 ( $19.40 \times 10^7$  cfu ml<sup>-1</sup>). However, significantly minimum growth of the test pathogen was recorded at pH 9.0 ( $2.50 \times 10^7$  cfu ml<sup>-1</sup>) followed by that at pH 8.0 ( $5.53 \times 10^7$  cfu ml<sup>-1</sup>) (Table 2).

Table 2: Effect of different pH levels on the growth of *Xanthomonas cucurbitae*

pH	Growth of bacterial isolates (×10 <sup>7</sup> cfu ml <sup>-1</sup> )	
	Bottle gourd	Pumpkin
4.0	0.00f (1.00)	0.00f (1.00)
5.0	25.33c (5.13)	19.40c (4.51)
6.0	44.16a (6.71)	27.03b (5.29)
7.0	36.15b (6.09)	31.62a (5.70)
8.0	6.80d (2.71)	5.53d (2.55)
9.0	4.16e (2.26)	2.50e (1.86)
CD0.05	0.33	0.35
SEm±	0.10	0.11

Figures in parentheses are square root transformed values

During present studies, pH levels 6.0 and 7.0 were found to be best whereas no growth of the pathogen was recorded at pH 4.0. These results were in accordance with Zhang and Babadoost (2018) who reported that optimum pH level for the growth of *Xanthomonas cucurbitae* ranged from 6.5 to 7.0 whereas, no growth was recorded at pH below 4.5. During the course of study, it was also observed that with the increase in pH level above 7.0, there was a decrease in the bacterial growth. These findings are supported by the findings of Suresh et al. (2013) who obtained maximum growth of *X. oryzae* pv *oryzae* at pH 7.0 and observed a decrease in growth of the bacterium with the increase in pH level of the medium.

3.3. Effect of different liquid media on the growth of *X. cucurbitae*

Among the six liquid media studied (Table 3) for the growth of pathogen, maximum growth of bottle gourd isolate was supported by nutrient glucose broth ( $9.31 \times 10^7$  cfu ml<sup>-1</sup>) followed by yeast extract dextrose CaCO<sub>3</sub> broth ( $8.11 \times 10^7$  cfu ml<sup>-1</sup>) and minimum growth of the bacterium was found in yeast extract nutrient broth ( $3.65 \times 10^7$  cfu ml<sup>-1</sup>). However in case of pumpkin isolate, maximum growth of the bacterium was supported by yeast extract dextrose CaCO<sub>3</sub> broth

Table 3: Effect of different liquid media on the growth of *Xanthomonas cucurbitae*

Liquid media	Growth of bacterial isolates ( $\times 10^7$ cfu ml <sup>-1</sup> )	
	Bottle gourd	Pumpkin gourd
Nutrient sodium chloride broth	7.70b	7.33cd
Nutrient glucose broth	9.31a	7.93bc
Yeast extract sucrose peptone broth	5.30c	8.71b
Nutrient yeast extract broth	6.13c	6.08d
Yeast extract dextrose CaCO <sub>3</sub> broth	8.11ab	10.63a
Yeast extract nutrient broth	3.65d	4.98e
CD ( $p=0.05$ )	1.27	1.35
SEm $\pm$	0.40	0.43

( $10.63 \times 10^7$  cfu ml<sup>-1</sup>) followed by nutrient sucrose peptone broth ( $8.71 \times 10^7$  cfu/ml) and nutrient glucose broth whereas, minimum growth was supported by yeast extract nutrient broth ( $4.98 \times 10^7$  cfu/ml).

Growth of both the isolates was well supported by yeast extract dextrose CaCO<sub>3</sub> broth as well as nutrient glucose broth while, yeast extract nutrient broth was least supportive for the growth of both the isolates of *X. cucurbitae* under study. As there are no reports in the literature regarding the effect of different nutrient media on the growth of *X. cucurbitae*, so these results cannot be compared with. However, many of the workers have used yeast extract dextrose CaCO<sub>3</sub> agar medium for studying the cultural characteristics of *X. cucurbitae* (Lamichhane et al., 2010; Babadoost and Ravanlou, 2012; Dutta et al., 2013 and Liu et al., 2016). Also, Jarial and Shyam (2004) while working on *X. campestris* pv *campestris* recorded a moderate growth of the bacterium on yeast extract dextrose calcium carbonate broth as well as nutrient glucose broth and minimum growth on yeast extract nutrient broth medium.

#### 4. Conclusion

The colonies of isolates of *Xanthomonas cucurbitae* infecting bottle gourd and pumpkin were mucoid, circular, smooth textured and yellow in colour with 2-4 mm diameter. Temperatures of 25 and 30 °C and pH levels of 6.0 and 7.0 were found to be optimum for the growth of bottle gourd and pumpkin isolates, respectively. Growth of both the isolates was well supported by yeast extract dextrose CaCO<sub>3</sub> broth as well as nutrient glucose broth.

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