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Symptomatic and morpho-cultural variability among different isolates of *Xanthomonas campestris* pv. *Mangiferaeindicae* causing mango bacterial leaf spot

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Abstract

Many commercial cultivars of mango are highly susceptible to bacterial leaf spot and infections can result in drastic yield losses. Symptomatic and morpho-cultural variability studied among different isolates of *Xanthomonas campestris* pv. *Mangiferaeindicae* causing mango bacterial leaf spot. Diseased samples collected from marathwada region expressed a wide range of variability in respect of lesion shape, size and colour etc. Cultural and morphological characters of different *Xanthomonas campestris* pv. *Mangiferaeindicae* isolates were studied on nutrient agar media. Eight isolates collected from different locations of Marathwada region differed in respect of cultural characteristics viz., pigmentation, colony shape, elevation, margin, surface appearance and cell shape were studied using NA as basal culture medium. Among the eight isolates tested, six isolates viz., Xcm1, Xcm3, Xcm4, Xcm6, Xcm7 and Xcm8 exhibited cream to white pigmentation while rest two (Xcm2 and Xcm5), exhibited white to pale yellow pigmentation. All the isolates had filiform colony shape, convex elevation and entire colony margin. However, all the isolates were morphologically single rods.

Keywords: *Xanthomonas campestris* pv. *Mangiferaeindicae*, bacterial leaf spot, mango, cultural morphological characters, nutrient agar

Introduction

Mango (*Mangifera indica*) is cultivated in most frost free tropical and warmer subtropical climates. It is the National fruit of India. Besides delicious taste, excellent flavour and attractive fragrance, it contains a variety of nutrients and rich in vitamin A & C. India ranks first in the production, consumption and export of mango all over the world with an area, production and productivity of 2262.8 000' ha, 19686.9 000' MT and 8.7 MT/ha respectively whereas, Maharashtra occupies an area of 157.07 ha, production 520.87 t and productivity of 3.58 mt/ha (Anonymous, 2019) [2]. Mango bacterial leaf spot disease which is also known as mango canker, bacterial spot, bacterial canker, black spot, mango blight, bacterial black spot is caused by *Xanthomonas campestris* pv. *Mangiferaeindicae* (Xcmi) (Gupta and Sharma, 2000) [7]. It is one of the most destructive bacterial disease of mango worldwide (Gagnevin and Pruvost, 2001) [6]. The disease is most serious in areas of high temperature (14-38 °C) and high rainfall (more than 1000 mm per year); during the growing season (Das, 2003).

Many commercial cultivars are highly susceptible to bacterial leaf spot and infections can result in drastic yield losses associated with premature fruit drop, reduction of fruit quality, and induction of severe defoliation especially when storms or hurricanes are involved. From 50 to 80% fruit infection is common on very susceptible cultivars.

Material and methods

Isolation of the bacterium

The bacterial leaf spot diseased mango plant specimens (leaves, fruits and branches) collected from mango orchards distributed in various agro- climatic zones of Marathwada region, were subjected first to ooze test to confirm association of the bacterium and them subjected to isolations on nutrient agar (NA) medium, by employing standard procedure. Upon completion of the incubation period, single colonies of the bacterium develop on NA was picked up aseptically, transferred on to fresh NA Petri plates and incubated 28 ± 2 °C, to obtain pure cultures of the bacterium isolates/strains. Applying same procedure, a total of eight isolates of *X. campestris* pv. *Mangiferaeindicae* were isolated. Pure cultures of the test bacterium strains were assigned the nomenclature, by considering the agro climatic zones and/or the mango plants from which day isolated. Pure culture thus obtained was preserved in refrigerator for further studies.

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Morpho-cultural characterization

Morpho-cultural characters of *Xanthomonas campestris* pv. *Mangiferaeindicae* pathogen was studied by microscopic and visual observation viz. cell shape, flagellation, colony edge, elevation, Pigmentation and surface appearance.

Result and discussions

1. Symptomatic variability of virulent pathogen in the field

During the collection of samples bacterial leaf spot symptoms expressed variability in respect of their lesion shape, size and colour etc.

Table 1: Symptomatic variability among the bacterial leaf spot specimens collected from different agro climatic zones of Marathwada region

| Isolate name | Agro climatic zone | Location | Symptoms | | |
|--------------|--------------------|-----------|-----------------------|------------------|------------------------------|
| | | | Lesion shape | Lesion size (mm) | Lesion colour |
| Xcm1 | SC | Anandwadi | Circular to irregular | 2 | Dark brown with yellow halo |
| Xcm2 | AR | Pachod | Circular to irregular | 2.5 | Brown with yellow halo |
| Xcm3 | AR | Antarweli | Circular to irregular | 2 | Dark brown |
| Xcm4 | AR | Kalamb | Circular to irregular | 2 | Light brown with yellow halo |
| Xcm5 | AR | Latur | Circular to irregular | 1 | Brown with yellow halo |
| Xcm6 | AR | Zari | Circular to irregular | 1 | Black |
| Xcm7 | MR | Mudkhed | Circular to irregular | 3 | Dark brown with yellow halo |
| Xcm8 | MR | Aundha | Circular to irregular | 2 | Brown with yellow halo |

Results (Table 1 and Plate I) revealed that lesion shape were circular to irregular on mango leaves, grown in the all agro-climatic zone of the Marathwada region. The size of the leaf lesion was also varied, ranged from 1 mm to 3 mm in dia., maximum lesion size was found in Mudkhed (Xcm7) isolate (3mm) from moderate rainfall zone followed by Antarweli (Xcm2), Pachod (Xcm1), Anandwadi (Xcm3), Kalamb (Xcm4) and Aundha i.e 2 mm from three agro-climatic zones of marathwada region. However, minimum lesion size was found in isolates Xcm5 and Xcm6 collected from latur and zari respectively Assured rainfall zone i.e 1 mm. Colour of lesion on mango leaves was mostly brown with yellow halo in agro-climatic zones viz., SC, AR, MR and dark brown with yellow halo in agro-climatic zones viz., AR and MR.

2. Morpho-cultural characters

Cultural characteristics viz., pigmentation, colony shape, elevation, margin, surface appearance and cell shape of different eight test isolates were studied using NA as basal culture medium. Cell shape was observed using binocular microscope (400X).

Results (Table 2, Plate II) revealed that, of the eight isolates tested, four isolates viz., Xcm1, Xcm4, Xcm5 and Xcm7 exhibited creamy white pigmentation, Xcm2 and Xcm6, exhibited Creamy white with pale yellow ting pigmentation while rest two isolates viz., Xcm3 and Xcm8 exhibited creamy white to pale yellow pigmentation. All the isolates had filiform colony shape, convex elevation and six isolates viz., Xcm1, Xcm3, Xcm5, Xcm6 and Xcm8 had entire colony margin and rest three isolates Xcm2, Xcm4 and Xcm7 had irregular margin.

Table 2: Morphological and cultural characteristics of *Xanthomonas campestris* pv. *Mangiferaeindicae* isolates

| Sr. No. | Isolates | Pigmentation | Elevation | Margin | Surface | Cell shape |
|---------|----------|-------------------------------------|-----------|------------------|-----------------------|-------------|
| 1 | Xcm1 | Creamy white | Convex | Entire margin | Smooth and glistening | Single rods |
| 2 | Xcm2 | Creamy white with pale yellow sting | Convex | Irregular margin | Smooth and glistening | Single rods |
| 3 | Xcm3 | Creamy white to pale yellow | Convex | Entire margin | Smooth and glistening | Single rods |
| 4 | Xcm4 | Creamy white | Convex | Irregular margin | Smooth and glistening | Single rods |
| 5 | Xcm5 | Creamy white | Convex | Entire margin | Smooth and glistening | Single rods |
| 6 | Xcm6 | Creamy white with pale yellow sting | Convex | Entire margin | Smooth and glistening | Single rods |
| 7 | Xcm7 | Creamy white | Convex | Irregular margin | Smooth and glistening | Single rods |
| 8 | Xcm8 | Creamy white to pale yellow | Convex | Entire margin | Smooth and glistening | Single rods |

Similar results were also reported earlier by many workers. (Ah-You *et al.*, 2007; Khalid and Sinha, 2008; Singh and Thind, 2014; Kharde *et al.*, 2018) [1, 8, 11, 9]. Manicom and Wallis (1984) [10] described that all strains isolated of *X. campestris* pv. *Mangiferaeindicae* were rod shaped and motile by means of single polar flagellum. On nutrient agar the cell was 0.4 to 0.5 by 1.0 to 1.1 μ m round, shallowly, convex colonies with entire margins were formed. The colour was initially smoke gray but soon became white to cream. With the age, the colonies became pale yellowish brown. Thirumalesh (2012) [12] reported that isolates of *X. campestris* pv. *Mangiferaeindicae* were motile and small rods singly or in pairs and produced white to yellow, mucoid, circular, raised 1

to 2 mm colonies on YNA and NA medium. Bandi (2019) [4] studied cultural variability of eight isolates of *X. campestris* pv. *Mangiferaeindicae*. The results revealed that the isolates differed with respect to colony characters such as size, shape, colour and appearance. Xcm1, Xcm2 and Xcm5 developed small to medium bacterial colonies and Xcm3, Xcm4, Xcm7 and Xcm8 developed medium to large colonies. The isolates Xcm1, Xcm3, Xcm5, Xcm6, Xcm7 and Xcm8 were having circular to irregular shape. Xcm2 and Xcm4 were having circular in shape. Isolates Xcm1, Xcm2, Xcm5 and Xcm7 were creamish white and isolates Xcm3, Xcm4, Xcm6 and Xcm8 formed creamish colonies.



Plate I: Symptomatic variability among the bacterial leaf spot specimens



| Isolates | Locations |
|----------|-----------|
| Xcm-1 | Anandwadi |
| Xcm-2 | Pachod |
| Xcm-3 | Antarweli |
| Xcm-4 | Kalamb |
| Xcm-5 | Latur |
| Xcm-6 | Zari |
| Xcm-7 | Mudkhed |
| Xcm-8 | Aundha |

Plate II: Pure culture of *X. campestris* pv. *Mangiferaeindicae*



Plate III: Morphocultural variability among the test isolates of *X. campestris* pv. *Mangiferaeindicae*

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