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Pathogenic variability among different isolates of *Xanthomonas campestris* pv. *Mangiferaeindicae* causing bacterial leaf spot of mango

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Abstract
Mango bacterial leaf spot, (*X. campestris* pv. *Mangiferaeindicae*) is one of the most devastating diseases throughout the world, affecting all kinds of commercial mango varieties. Pathogenic variability among the eight isolates of *Xanthomonas campestris* pv. *Mangiferaeindicae* collected from different agro climatic zones of Marathwada region was detected by two separate methods *viz.*, attached leaf assay and detached leaf assay. Eight different isolates collected from Marathwada region were subjected to pathogenic variability in attached leaf assay under screen house condition. Entire eight test isolates of *X. campestris* pv. *Mangiferaeindicae* found pathogenic and caused bacterial leaf spot in mango (Kesar). Average incubation period (days) varied from 12.33 (AR) to 14 (MR). In detached leaf technique under controlled lab conditions, leaves of total six varieties of mango *viz.*, Local, Kesar, Dasheri, Neelam, Amrapali and Alphonso were used and observation were recorded on incubation period and symptom type. The entire eight test isolates of *X. campestris* pv. *Mangiferaeindicae* found pathogenic and caused bacterial leaf spot in leaves of all mango cultivars However, Local and Kesar cultivar showed moderate and more symptoms of leaf spot, while rest all cultivars *viz.*, Dasheri, Neelam, Amrapali and Alphanso showed less symptoms of leaf spot.

Keywords: *Xanthomonas campetris* pv. *Mangiferaeindicae*, bacterial leaf spot, mango, Pathogenic variability

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). It is one of the most destructive bacterial disease of mango worldwide (Gagnevin and Pruvost, 2001) [8]. The disease is most serious in areas of high temperature (14-38 ^{0}C) and high rainfall (more than 1000 mm per year); during the growing season (Das, 2003) [13]. 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
1. Attached leaf assay (Francis *et al.* 2010) [7]
Mature leaves on greenhouse seedlings of mango of the same type used for detached leaf assay were inoculated using needleless syringe. Test bacterial inoculum (as described under detached leaf assay) was infiltered by pressing needleless syringe to produce a zone of water-soaked tissue about 6mm in diameter. Three injection infiltrations were performed on each side of the midvein. Three leaves were inoculated per plant, and three plants were inoculated per assay. The inoculated shoots were covered with a plastic bag for 24 h to maintain high humidity conductive for bacterial growth in the leaves. Developments of symptoms on leaves were evaluated periodically up to 21 days post inoculation.

2. Detached leaf assay (Yin *et al*. 2011) [14]

A single bacterial colony was collected from the test bacteria cultured on NA medium and inoculated in NA broth for mass multiplication. The resultant culture was kept at 28 ^{0}C and shaken overnight at 200 rpm, which was collected and resuspened in the medium to the final concentration of 10^8cfu/ml before inoculation. Two third to fully expanded, mature leaves from mango susceptible year old cultivar of Kesar seedlings, grown in green house was detached and brought in to laboratory. Mango leaves were first washed with distilled water and then subjected to inoculation on abaxial side using an insect pin (0.5mm in diameter). Four inoculation sites (each composed of 5 pricks) were made on both surface of midvein, onto which an aliquot of 10µl of the bacterial suspension was dropped with the help of micropipette. Following the inoculation, the leaves were placed above wet filter paper in Petri dish, which were sealed with parafilm to sustain high humidity. Two leaves were taken for inoculation of per isolate. The petri dishes were maintained at 28^{0}C in incubator for up to 21 days. At various time points, occurrence of symptoms was scored.

Result and discussions

Pathogenic variability

The pathogenic variability among the eight isolates of *X. campestris* pv. *Mangiferaeindicae* bacteria *viz.,* Xcm1, Xcm2, Xcm3, Xcm4, Xcm5, Xcm6, Xcm7 and Xcm8 collected and isolated from different agro-climatic zones of Marathwada region was detected by two separate methods *viz.,* attached leaf assay and detached leaf assay.

Attached leaf assay

The pathogenic variability among the eight isolates of *X. campestris* pv. *Mangiferaeindicae* were studied by attached leaf technique under screen house condition and susceptible variety of mango *i.e* Kesar was used.

The results (Table 1, Plate I and Fig. 1) revealed that, all eight isolates of *X. campestris* pv. *Mangiferaeindicae* were pathogenic and caused bacterial leaf spot in mango (Kesar). Average incubation period varied from 12 (Xcm2) to 14 (Xcm7) days on mango among eight isolates. Maximum incubation period was recorded in isolate Xcm7 from zone MR (14). However, in all seven isolates, the period varied non significantly from 12.00 to 13.33 days. The number of spots on leaves also varied from 2.66 to 4 in numbers. Significantly more numbers of spot found in Xcm8 isolate (4.00) from agro-climatic zone MR, followed by lesser number of spots in rest of the isolates varied non significantly from 2.66 to 3.76.

Size of leaf spot also varied significantly ranging from 1.66 to 2.5 mm in diameter. There was no significant difference in spot diameter in any of the isolates. On the basis of results it has been inferred that the mango bacterial incubation period, number of spot and size were varied on Kesar cultivar due to isolates collected from different regions of Marathwada falling under various agro-climatic conditions it may be due different genetic makeup of isolates.

Table 1: Pathogenic variability among the *Xcm* isolates (attached leaf assay)

Sr. No.	Isolates	Agro climatic Zone	Av. Incubation period (Days)*	Av. No. of spots*	Av. Size of spot (mm)*
1	Xcm1	SC	12.66	3	2
2	Xcm2	AR	12	2.66	1.66
3	Xcm3	AR	12.66	2.76	1.76
4	Xcm4	AR	13.33	3	1.83
5	Xcm5	AR	12.33	3.66	2.26
6	Xcm6	AR	13	3.33	1.83
7	Xcm7	MR	14	3.76	2.5
8	Xcm8	MR	13.33	4	2.16
S.E. ±			0.47	0.37	0.28
C.D. (P= 0.01)			1.38	1.09	0.84

*Mean of three replications

Similar results were also reported earlier by many workers. Tsuchiya *et al.,* (2003) [13] tested pathogenecity using two year old plants. The tests were done on whole plants using an automizer where young green twigs and mature green leaves were wounded with needles and sprayed with inoculums prepared by standardizing cell suspensions about 10^8 cfu/ml. The plants were covered with polyethylene bags and kept in greenhouse at 20^0 - 28 ^{0}C under natural light. Bhure *et al.,* (2019) [4] studied *in vivo* pathogenic variability by Pot culture (seedling inoculation) technique among ten isolates of *X. axonopodis* pv. *citri* which were isolated from different regions of Vidarbha. Results showed that all isolates exhibited symptoms between ranges of 15 (Xac 10) to 25 DAI *in vivo* and conformed that isolate Xac-10 showed highly pathogenic to initiate water soaked lesion and fully developed symptoms after 15 days under *in vivo* condition. While isolate Xac-10 (Akola) gave 3 mm water soaked lesions surrounded by yellow halo zone; whereas Xac-2, Xac-3, Xac-5, Xac-6 and Xac-7 were found weak canker lesions and Xac-1, Xac-4, Xac-8 and Xac-9 were found moderate canker lesions on leaves under *in vivo* condition.

Detached leaf assay

The pathogenic variability among the eight isolates of *X. campestris* pv. *Mangiferaeindicae* was studied by detached leaf technique *in vitro* condition on different varieties of mango. The pathogenic variability among eight isolates of *X. campestris* pv. *Mangiferaeindicae* bacteria *viz.,* Xcm1, Xcm2, Xcm3, Xcm4, Xcm5, Xcm6, Xcm7, and Xcm8 were studied *in vitro* by detached leaf technique under controlled conditions and leaves of total six varieties of mango *viz.,* Local, Kesar, Dasheri, Neelam, Amrapali and Alphanso were used.

Results (Table 2, Plate II and Fig. 2) revealed that, average incubation period (days) varied from 9.00 (Xcm1) to 16.33 (Xcm6) on different cultivars of mango among the eight isolates. However, average incubation period (days) varied from 9.33 (Xcm3) to 11.33 (Xcm7) on Local cultivar of mango among eight isolates. Maximum incubation period (days) was in isolate Xcm7 (11.33) followed by Xcm4 (11.13), Xcm15 (10.66), followed by two isolates *viz.,* Xcm6 and Xcm8 exhibited same incubation period *i.e* 10.33, followed by Xcm2 (10.00), and Xcm1 (9.99), while minimum

average incubation period (days) was in Xcm3 (9.33). On Kesar cultivar of mango among eight isolates, maximum incubation period (days) was in isolate Xcm8 (10.66) followed by two isolates *viz.,* Xcm2 and Xcm7 exhibited same incubation period *i.e* 10.33, followed by Xcm4 (10.00) while, minimum average incubation period (days) was in Xcm1 (9.00) followed by Xcm5 (9.33), followed by two isolates *viz*., Xcm3 and Xcm6 again exhibited same incubation period (9.66). On Dasheri cultivar of mango among eight isolates, maximum incubation period (days) exhibited by Xcm8 (13.66) followed by Xcm7 (13.33), Xcm4 (13.11), Xcm6 (12.99), Xcm3 (12.66) while, minimum average incubation period (days) was in Xcm1 (10.66) followed by Xcm2 (12.33) and Xcm5 (11.66). On Neelam cultivar of mango among eight isolates, maximum incubation period (days) was in Xcm8 (14.66) followed by Xcm7 (14.33), Xcm5 (14.11), Xcm4 (13.66), Xcm3 (13.33) while, minimum average incubation period (days) was in Xcm6 (11.99) followed by Xcm2 (12.33) and Xcm1 (12.66). On Amrapali cultivar of mango among eight isolates, maximum incubation period (days) was in isolates Xcm8 (14.99) followed by Xcm2 (14.66), followed by two isolates *viz*., Xcm1 and Xcm5 *i.e* 14.33, followed by Xcm7 (14.13), Xcm3 (13.99) while, minimum average incubation period (days) was in two isolates *viz.,* Xcm4 and Xcm6 *i.e* 13.66. On Alphanso cultivar of mango among eight isolates, maximum incubation period (days) was in isolate Xcm6 (16.33), followed by Xcm5 (16.13), followed by two isolates exhibited same incubation period *viz.,* Xcm1 and Xcm4 *i.e* 15.66, followed by Xcm2 (15.33) while minimum incubation period (days) was in two isolates Xcm3 and Xcm8 exhibited same incubation period *i.e* 14.99 followed by Xcm7 (15.11) and Xcm2 (15.33).

Symptoms exhibited by different isolates were grouped under four categories viz., (-) no leaf spot, (+) less leaf spot, (++) moderate leaf spot and (+++) more leaf spot. Different pattern of symptom expression was found in all the isolates among six cultivars. Kesar and Local cultivar of mango showed moderate to severe bacterial leaf spot, while rest all cultivars *viz.,* Dasheri, Neelam, Amrapali and Alphanso showed lesser leaf spots. From the ongoing results it has been inferred that the mango bacterial incubation period, frequency of spots and size varied from different cultivars due to isolates collected from different regions of Marathwada falling under three agro- climatic zones, it may be due to difference in virulence of isolates.

Similar results were also reported earlier by many workers. (Moffett *et al.,* 1977; Tsuchiya *et al.,* 2003; Sain *et al.,* 2008; Al-Saleh *et al.,* 2014) [10, 13, 11, 1]. Dayakar and Gnanamanickam (1996) [6] studied the pathogenic variability among 20 isolates collected from various locations of Southern India on six local commercial mango varieties and the bacterial suspension was artificially inoculated on mango leaves. The lesion size was measured after 10 days of inoculation and some strains were more aggressive on susceptible varieties. Tsuchiya *et al.,* (2003) [13] tested the pathogenecity of *X. campestris* pv. *Mangiferaeindicae* on detached leaves of mangos. Detached leaves were aseptically inoculated with 50 µl inoculum (10^8 cfu/ml) and were placed at 25^0 C under fluorescent light. Symptom development were observed after a week of inoculation. Sanahuja *et al.,* (2016) [12] proved the pathogenecity of *X. campestris* pv. *Mangiferaeindicae* strains by infiltration methods. Where mango varieties Kiett and Haden plants were injected with 18 hrs. Old YGPA colonies ($1x10^5$ cfu/ml). After seven days, black lesions developed on inoculated leaves.

Table 2: Pathogenic variability among the *Xcm* isolates (detached leaf assay)

SN	Isolates	Agro-climatic Zones	Reaction on leaves of different cultivars after 20 days of inoculation											
			Local		Kesar		Dasheri		Neelam		Amrapali		Alphanso	
			Incubation Period	Symptoms	Incubation Period	Symptoms	Incubation Period	Symptoms	Incubation Period	Symptoms	Incubation Period	Symptoms	Incubation Period	Symptoms
1.	Xcm1	SC	9.99	+++	9.00	+++	10.66	++	12.66	+	14.33	+	15.66	+
2.	Xcm2	AR	10.00	++	10.33	++	12.33	+	12.33	+	14.66	+	15.33	+
3.	Xcm3	AR	9.33	+++	9.66	+++	12.66	++	13.33	+	13.99	+	14.99	+
4.	Xcm4	AR	11.13	++	10.00	+++	13.11	+	13.66	+	13.66	+	15.66	+
5.	Xcm5	AR	10.66	++	9.33	+++	11.66	+	14.11	+	14.33	+	16.13	+
6.	Xcm6	AR	10.33	+++	9.66	+++	12.99	+	11.99	+	13.66	+	16.33	+
7.	Xcm7	SC	11.33	++	10.33	++	13.33	+	14.333	+	14.13	+	15.11	+
8.	Xcm8	SC	10.33	+++	10.66	++	13.66	+	14.66	+	14.99	+	14.99	+

- No symptoms of leaf spot, + less symptoms of leaf spot, ++ moderate symptoms of leaf spot, +++ more symptoms of leaf spot

Plate 1: Pathogenicity test (Attached leaf assay)

Plate I: Pathogenicity test (detached leaf assay)

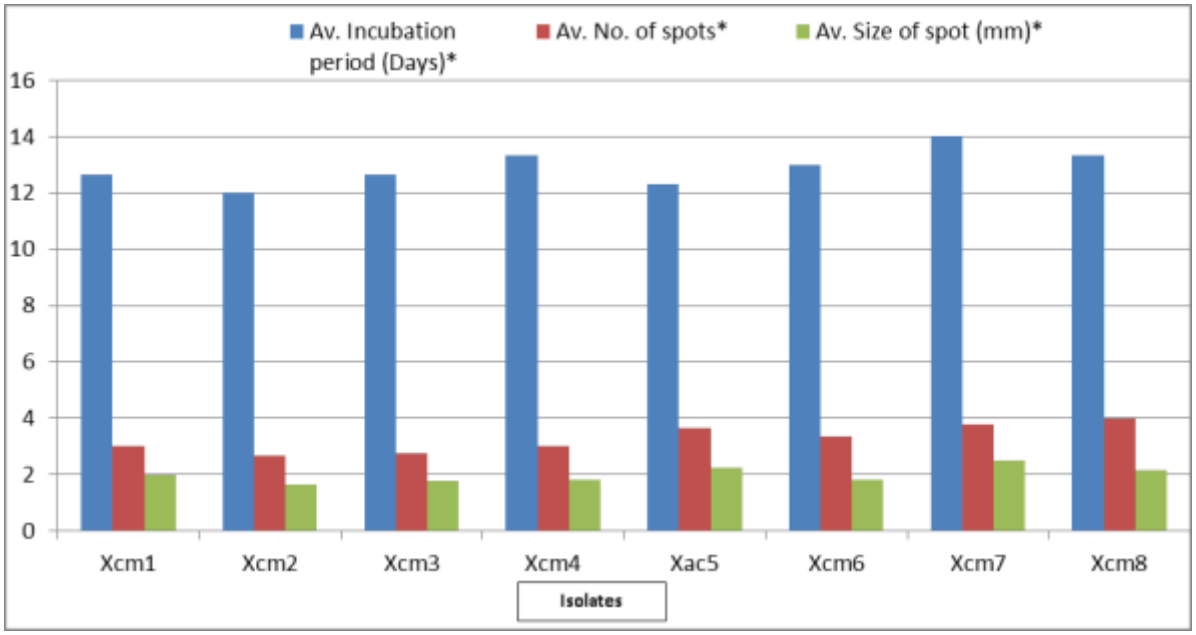

Fig 1: Pathogenic variability among the *Xcm* isolates (attached leaf method)

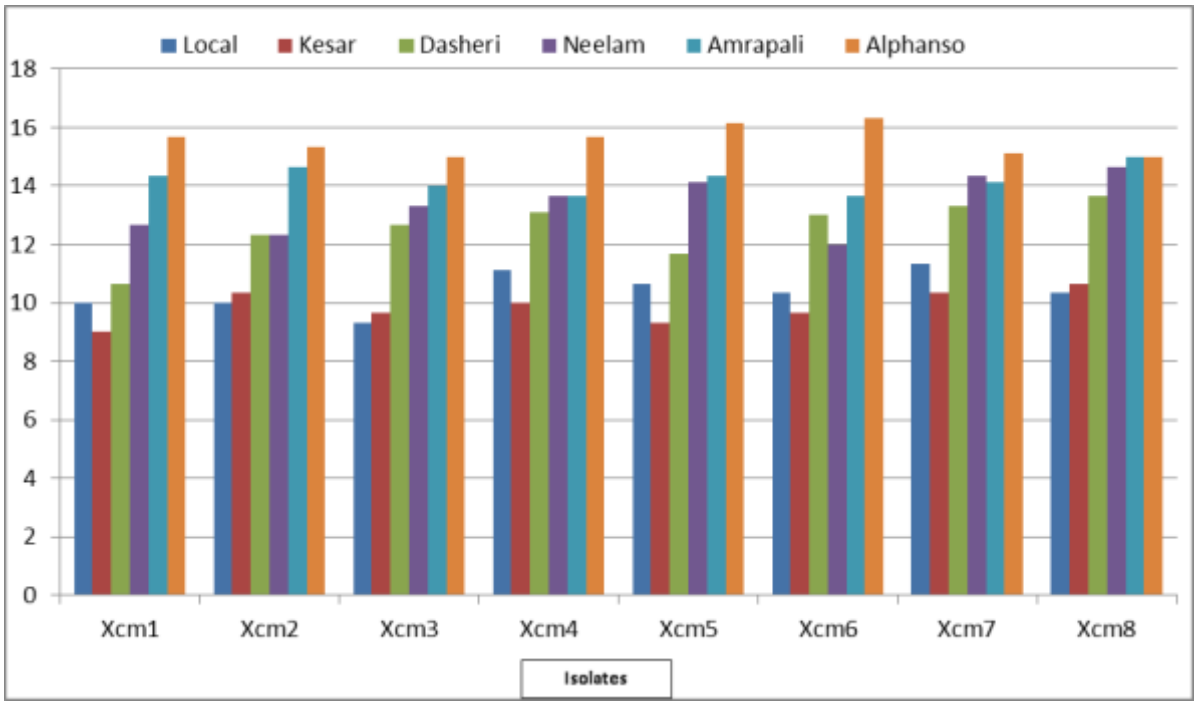

Fig 2: Pathogenic variability among the *Xcm* isolates (detached leaf method)

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