



## Antibacterial Activity of Leaf Extract of *Azadirachta Indica* Against *Xanthomonas Campestris* P.v. *Mangiferaeindicae*

### KEYWORDS

Antibacterial activity, *Xanthomonas campestris* pv. *mangiferaeindicae*, *Azadirachta indica*

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**ABSTRACT** *Mango bacterial canker disease (MBCD) caused by Xanthomonas campestris pv. mangiferaeindicae (Xcmi) is one of the important diseases of mango affecting a number of commercial cultivars. The pathogen affects different plant parts like leaf, stem and fruit. Favorable environmental conditions cause severe loss to the crop. Leaf extract of various plants were tested against Xcmi for antibacterial activity. Azadirachta indica is well known for its antimicrobial activity. Hence, leaf extracts of A. indica assessed for its antibacterial activity against 25 strains of Xcmi collected from different parts of Marathwada region of Maharashtra. In vitro studies have been performed by using cup-plate method to examine the activity. Fresh leaf extracts of A. indica plants were screened against 25 strains of Xcmi. The maximum activity was recorded against Xcmi.09 (Mean activity zone – 21.86 mm) followed by Xcmi.07 (Mean activity zone – 21.55 mm) and minimum against Xcmi.23 (Mean activity zone – 18.30 mm) strain under investigation. The ultimate aim of the research work was to develop economically and technically viable field formulations for the farmers, which will be Bio-ecologically compatible for management of plant bacterial diseases.*

### INTRODUCTION

Bacterial diseases of fruit plants are known to cause great damages all over the world. Mango (*Mangifera indica* L.) is the most ancient among the tropical fruits. Among the bacterial diseases, bacterial canker is the most severe disease on Mango, which is caused by *Xanthomonas campestris* pv. *mangiferaeindicae* (Xcmi). The pathogen affects different plant parts like leaf, stem and fruit. Fruit cracking due to the disease causes extensive loss to the cultivator.

For the management plant diseases, various chemicals are used since last several years, all over the world. They tend to accumulate in animal tissues posing threat to human health. Green plants represent a reservoir of effective chemotherapeutics and can provide valuable sources of natural pesticides (Balandrin et al., 1985; Hostettmann & Wolfender, 1997). Medicinal properties of leaf extracts have been reported by many workers (Naik, 1998). The medicinal properties of leaf extracts have also been mentioned by Kirtikar and Basu (1991). Antibacterial activity of various medicinal plants were assessed and observed that *Azadirachta indica*, member of Meliaceae family showed good antibacterial activity against Xcmi strains.

Various studies reciprocate that it contains active substances with multiple medicinal properties (Bhuiyan et al., 1997). It has been used in large quantity in the pharmaceutical and cosmetic industries. Badam et al., (1999) reported antiviral and virucidal effects of methanolic extract fraction of leaves of neem. Antifungal activity to treat infections with dermatophytic fungi has been reported by Radhika and Michael (2013).

Antibacterial activity of *A. indica* against skin pathogen was reported by Mule (2012). Sarmiento et al. (2011) explained antibacterial effect of *A. indica* leaf extracts on *Staphylococcus aureus*. Various workers recorded antibacterial activity of leaf extracts of *A. indica* against number of bacterial pathogens (Mishra et al., 2013; Rajasekaran et al., 2008; Maragathavalli et al., 2012). However, during this research work antibacterial activity of leaf extract of *A. indica* has been assessed against 25 strains of Xcmi to observed the behavior of these strains.

### MATERIALS AND METHODS

The strains of causal organism of MBCD i.e. Xcmi were collected from Marathwada region of Maharashtra. Diseased Mango samples were collected from various districts (Aurangabad, Jalna, Hingoli, Nanded, Parbhani, Beed, Latur and Osmanabad) of Marathwada region and brought to the

laboratory for further investigation. Studies were performed using these samples and maintained various 25 Xcmi strains on Nutrient Agar (NA) medium.

#### a) Preparation of leaf extract:

The leaves of the plants were collected, thoroughly washed with tap water and then rinsed with sterile distilled water. They were dried in shade until all moisture evaporated. These leaves were powdered by using electric grinder and packed into polythene bags. One gm of the powder was taken and added to 10 ml of sterile distilled water. Then it was subjected to ultracentrifuge for 20 min at  $-4^{\circ}\text{C}$  at the 11000 rpm (Pawar & Papdiwal, 2010). This extract was used for the further studies.

#### b) Cup Plate Method:

It is a method of testing antibacterial activity. For this, the bacterial suspension was prepared by adding 10 ml sterile distilled water to 2 days old NA slope culture. Five drops of bacterial cell suspension were poured in sterilized petridishes (9 cm diameter) onto which 20 ml of nutrient agar was poured and slowly mixed and then allowed to solidify (Pawar & Papdiwal, 2012).

In the centre of the medium, a cup cavity of 8 mm diameter was made with sterilized No. 4 cork borer. This cup was filled with 0.1 ml of the leaf extract. The petridishes were incubated for 24 hrs at  $25\pm 2^{\circ}\text{C}$  and the observations were recorded as diameter of inhibitory zone in mm. Diameter of the activity zone was measured in 3-4 angles and mean was considered for accuracy. Cup cavity filled with sterile distilled water was used as control in all the experiments. All experiments were repeated for four times (Experiment. A, B, C & D).

### RESULTS AND DISCUSSION

It was observed from table 1 that *A. indica* showed antibacterial activity against all 25 strains of Xcmi under investigation. The maximum activity was recorded against Xcmi.09 (Mean activity zone – 21.86 mm) followed by Xcmi.07 (Mean activity zone – 21.55 mm) and minimum against Xcmi.23 (Mean activity zone – 18.30 mm) strain under investigation. Average activity of all Xcmi strains was 19.61 mm. Activity of *A. indica* ranges between 18 to 22 mm. (Fig. 01). Eleven Xcmi strains (Xcmi.06, Xcmi.07, Xcmi.08, Xcmi.09, Xcmi.12, Xcmi.13, Xcmi.15, Xcmi.18, Xcmi.21, Xcmi.24, Xcmi.25) have showed more activity than average activity of all strains; while Fourteen Xcmi strains (Xcmi.01, Xcmi.02, Xcmi.03, Xcmi.04,

Xcmi.05, Xcmi.10, Xcmi.11, Xcmi.14, Xcmi.16, Xcmi.17, Xcmi.19, Xcmi.20, Xcmi.22, Xcmi.23) showed less activity.

Similar results were recorded by Thirumalesh et al., (2012) against *X. campestris* pv. *mangiferaeindicae*. They have reported antibacterial activity of crude extracts of eight plants including *A. indica*, by using solvents like petroleum ether, chloroform, methanol and water against *X. campestris* pv. *mangiferaeindicae*. Rajasekaran et al., (2008) studied antibacterial activity of *A. indica* against various bacteria viz. *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus cereus*, *Micrococcus glutamicus*, *Lactobacillus bulgaris*, and *Staphylococcus aureus*. Mule (2012) concluded that leaves of Neem may be useful against skin pathogens like *Streptococcus pyogenes* and *Staphylococcus aureus*. Maragathavalli et al.,(2012) evaluated antimicrobial activity of the chemical compounds obtained from Neem leaves.

### CONCLUSION

It was observed from the research work, that leaf extract of *A. indica* is effective against all the strains of Xcmi. The leaf extract is eco-friendly, economic and technically viable field formulation, which will be Bio-ecologically compatible for management of various strains of Xcmi.

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**Table 01: Antibacterial Activity of Azadirachta indica against Xcmi strains**

Sr. No	Name of the Xcmi strain	Azadirachta indica Antibacterial activity: Mean Zone of Inhibition (in mm)				
		Exp. A	Exp. B	Exp. C	Exp. D	Mean
1.	Xcmi.1	19.34	19.10	18.84	19.22	19.13
2.	Xcmi.2	18.52	18.22	18.90	18.40	18.51
3.	Xcmi.3	19.88	19.10	19.93	19.01	19.48
4.	Xcmi.4	18.90	19.21	19.15	19.12	19.10
5.	Xcmi.5	18.86	18.45	18.52	18.11	18.49

6.	Xcmi.6	20.12	20.36	20.56	20.60	20.41
7.	Xcmi.7	21.25	21.63	21.40	21.90	21.55
8.	Xcmi.8	19.52	20.10	20.05	19.85	19.88
9.	Xcmi.9	22.10	21.94	21.45	21.95	21.86
10.	Xcmi.10	18.75	18.80	18.57	18.60	18.68
11.	Xcmi.11	18.68	19.10	18.82	19.00	18.90
12.	Xcmi.12	19.45	19.82	19.59	19.91	19.69
13.	Xcmi.13	19.84	20.45	20.47	20.72	20.37
14.	Xcmi.14	18.64	18.75	19.11	19.05	18.89
15.	Xcmi.15	19.86	19.98	20.17	20.10	20.03
16.	Xcmi.16	18.22	18.36	18.40	18.28	18.32
17.	Xcmi.17	19.35	19.54	18.77	18.86	19.13
18.	Xcmi.18	19.83	20.45	20.44	20.67	20.35
19.	Xcmi.19	19.14	19.75	19.58	19.73	19.55
20.	Xcmi.20	19.28	19.22	19.00	19.10	19.15
21.	Xcmi.21	20.47	20.83	20.00	20.79	20.52
22.	Xcmi.22	18.99	19.55	19.08	18.95	19.14
23.	Xcmi.23	18.50	18.38	18.10	18.22	18.30
24.	Xcmi.24	20.13	20.00	19.88	20.07	20.02
25.	Xcmi.25	21.42	20.50	20.75	20.48	20.79

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