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# *In vitro* Evaluation of Different Chemicals and Plant Extract against *Xanthomonas campestris* pv*. mangiferae indicae*

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***Abstract*:** Different chemicals, plant extracts and two natural products were evaluated *in-vitro* against the *Xanthomans campestris* pv. *mangiferaeindicae* at different concentrations by using paper disc method. Seven different chemicals were evaluated at different concentrations of 500, 1000, 1500 and 2000 ppm including chitosan at 125, 250, 375 and 500 ppm with low, medium and high molecular weight. Chitosan with high molecular weight gave the maximum inhibition zone (51.44 mm) at 500 ppm followed by medium molecular weight chitosan at same concentration and high molecular weight chitosan at 375 ppm concentration whereas, minimum inhibition zone was in captan at 2000 ppm. Various plant extracts and natural products were also evaluated at different concentration of 5,10,15 and 20 per cent. Out of which, only *Eucalyptus hybrida* proved to be effective in inhibiting the growth of bacterium. Maximum diametric inhibition zone (1.85 mm) was at 20 per cent concentration and minimum was at 5 per cent concentration.

***Keywords*:** *Xanthomonas campestris* pv. *mangiferaeindicae*, Chemicals, Plant extracts, Chitosan, *Euclyptus hybrida*

Mango (*Mangifera indica* L.) belonging to family Anacardiaceae is commercially the most important fruit crop of India and consists of around 30 species of tropical fruit tree (Shah et al 2010). Mango is produced throughout the country with states in all regions contributing significantly to total output. Mango is grown worldwide with production of 55.4 MT (Anonymous 2019). In India, area under cultivation for mango production is 2291 thousand ha with production of 20444 thousand MT (Anonymous 2020). From seedling through fruiting in storage or transit, mango is susceptible to a variety of diseases. Anthracnose, black tip, bacterial leaf spot, dieback, mildew, sooty mould and phoma blight are among the diseases that growers in India are concerned about (Prakash 2007). In 1909, first documented the *Bacillus mangiferae* caused bacterial black spot of mango in South Africa (Doidge 1915). In India, Patel et al (1948) identified the disease as bacterial leaf spot of mango in Poona and Dharwar, and named the pathogenic bacterium as *Pseudomonas mangiferaeindicae*. Robbs et al (1974) proposed the current name of *Xanthomonas campestris* pv. *mangiferaeindicae*. *X. campestris* pv. *mangiferaeindicae* causes mango bacterial leaf spot disease, also known as mango canker, bacterial spot, bacterial canker, black spot, mango blight, or bacterial black spot (Gupta and Sharma 2000).

Mango bacterial black spot is very difficult to control and it usually becomes a limiting factor for mango industries when fungal diseases and other pests can be managed at acceptable levels. This is one of the most destructive bacterial disease of mango worldwide (Gagnevin and Pruvost 2001). Leaves of mango showed typical symptoms of bacterial leaf spot, formed lesions which were black, slightly raised, angular and sometimes produced a chlorotic halo. Later in the season, fruit symptoms consisted of small water-soaked spots around lenticels that later changed into black star shaped erumpent lesions. Moreover, cankers on twig were also observed sporadically (Zombre et al 2017). It results in 10 to 70 per cent fruit drop, 10 to 85 per cent loss in fresh yield and 5 to 100 per cent losses in storage all over the world (Haggag 2010).

The most effective strategy to control the disease is to prevent it from spreading to new places by enforcing tight quarantine restrictions. Various chemicals including an antibiotic, different fungicides and plant extracts were evaluated against *X. campestris* pv. *mangiferaeindicae* to manage the pathogen. Streptocycline was reported effective against the disease (Thirumalesh et al 2012, Tejaswini 2019). Chitosan is a new natural polymer which is nowadays become effective against various bacterial disease and having efficient antibacterial agent (Coqueiro and Di Piero 2011) which control different strains of *Xanthomonas.* There is no literature cited for chitosan and *Euclyaptus hybrida* against *X. campestris* pv. *mangiferaeindicae.* Hence, so the recent studies on various chemicals and plant extract was conducted.

## MATERIAL AND METHODS

The present investigation was carried out in the research laboratory, Department of Plant Pathology, College of Horticulture and Forestry Neri, Hamirpur during the year 2019-2021.

***In vitro* evaluation**: In all, seven chemicals, seven plant extracts and two natural products were evaluated *in-vitro* against the pathogen isolates by paper disc method (Loo et al 1945). For this, 1 ml of 72 h old bacterial suspension was mixed with molten NSA (20 ml) in sterile Petriplate. Paper discs (5mm) soaked in each concentration of chemical was placed in Petriplate already plated with NSA and bacterial suspension. These petriplates were then incubated at 28±2ºC after 48 h and data on diametric inhibition zone (mm) were recorded.

**Evaluation of chemicals against *X. campestris* pv. *mangiferaeindicae:*** In all, seven chemicals including natural polymer (Chitosan) were evaluated for their efficacy against *X. campestris* pv. *mangiferaeindicae* under *in vitro* conditions by paper disc method. All chemicals were evaluated at four different concentrations (Table 1).

**Evaluation of plant extracts/ natural products against *Xanthomonas campestris* pv. *mangiferaeindicae*:** Extracts of dried leaves of *Azadirachta indica* (Neem), *Eucalyptus hybrida* (Safeda), *Murraya koenigii* (Curry Leaf), *Lantana camera, Justicia adhatoda* (Basuti), *Cannabis sativus* (Bhang) and *Calotropis gigantean* (Aak) and natural product (10 days old sour butter milk and cow urine) were evaluated for their inhibitory activity against *X. campestris* pv. *mangiferaeindicae* by paper disc inhibition zone method. In order to obtain various concentrations such as 5, 10, 15 and 20 per cent weight/volume of extracts, 100 g of plant material was crushed and soaked in 300 ml distilled water. The mixture was then boiled to reduce the volume to 1/3$^{rd}$ of original *i.e.* to get 100ml as final volume of the extract. This extract served as 100 per cent concentration. The contents were then filtered through double layered muslin cloth so as to remove debris. These extracts were then autoclaved at 15 p.s.i. pressure at 121ºC for 20 minutes. Final desirable concentration of the extract was obtained by adding desired amount of sterilized distilled water to the extracts. The paper discs were soaked in each concentration of extract as mentioned earlier. In case of natural product, 10 days old sour butter milk and cow urine itself served as 100 per cent concentration which was further adjusted to desired concentration by adding sterilized distilled water. All the plant extracts,10 days old sour butter milk and cow urine were evaluated at four concentrations *viz.,* 5, 10, 15 and 20 per

**Table 1**. *In vitro* effect of different chemicals against *X. campestris* pv. *mangiferaeindicae*

| Chemicals | Diametric inhibition zone (mm) at different concentration (ppm) | | | | Overall mean |
|---|---|---|---|---|---|
| | 500 | 1000 | 1500 | 2000 | |
| Streptocycline* | 22.50 (28.30) | 23.78 (29.17) | 25.78 (30.50) | 27.23 (31.44) | 24.82 (29.85) |
| Copper hydroxide | 8.56 (16.94) | 11.83 (20.11) | 14.22 (22.15) | 15.78 (23.39) | 12.60 (20.65) |
| Captan | 6.45 (14.70) | 8.00 (16.41) | 9.89 (18.32) | 10.33 (18.74) | 8.67 (17.04) |
| Bordeaux mixture** | 18.78 (25.67) | 20.67 (27.03) | 21.33 (27.50) | 22.67 (28.42) | 20.86 (27.15) |
| Cuperous oxide | 0.00 (0.00) | 7.33 (15.70) | 8.00 (16.42) | 9.44 (17.88) | 6.12 (12.50) |
| Low molecular weight chitosan*** | 28.22 (32.08) | 35.00 (36.26) | 38.22 (38.17) | 44.22 (41.67) | 36.42 (37.04) |
| Medium molecular weight chitosan*** | 31.11 (33.89) | 41.22 (39.93) | 43.11 (41.02) | 48.89 (44.35) | 41.08 (39.79) |
| High molecular weight chitosan*** | 34.22 (35.79) | 45.33 (42.30) | 47.78 (43.71) | 51.44 (45.81) | 44.69 (41.90) |
| Copper oxychloride | 7.33 (15.70) | 7.89 (16.30) | 10.11 (18.52) | 11.67 (19.96) | 9.25 (17.62) |
| Overall mean | 18.13 (22.56) | 22.34 (27.02) | 24.27 (28.48) | 26.85 (30.18) | |

| $C.D_{p\geq0.05}$ | $S.E_{(d)}$ |
|---|---|
| Treatment | 0.55 | 0.28 |
| Concentration | 0.37 | 0.18 |
| Treatment ×Concentration | 1.01 | 0.55 |

Figures in parentheses are angular transformed values, * indicates the concentrations were 50, 100, 150 and 200 ppm. ** indicates the concentrations were 1500, 2000, 2500 and 3000 ppm,*** indicates the concentrations were 125, 250, 375 and 500 ppm.

cent. Simultaneously, a check treatment was maintained in which the paper discs were soaked in sterilized distilled water instead of plant extract. Data on diametric inhibition zone (mm) of the pathogen were recorded after 48 h of incubation at 28±2ºC.

**Data analysis:** The laboratory experiments were conducted with 3 replications while, results were statistically analyzed by using and online software OPSTAT (Sheoran 2006).

**RESULTS AND DISCUSSION**

**Evaluation of different chemicals against *X. campestris* pv. *mangiferaeindicae:*** The significantly maximum mean diametric inhibition zone (44.69 mm) was in high molecular weight chitosan treatment (Table 1, Plate 1) followed by medium and low molecular weight chitosan and streptocycline that the significantly maximum (51.44 mm) zone of inhibition was recorded when high molecular weight chitosan at 500 ppm followed by medium molecular weight chitosan (at same concentration and high molecular weight chitosan at 375 ppm concentration. Significantly minimum (6.45 mm) zone of inhibition was in captan followed by copper oxychloride and copper hydroxide at 500 ppm. However, no inhibition was observed in cuperous oxide at 500 ppm. An intermediate zone of inhibition was recorded in rest of the chemicals evaluated at different concentrations. There is no literature cited for the chitosan against *X. campestris* pv. *mangiferaeindicae*. The result proved that chitosan was the most effective chemical against the pathogen. To further support our study, different workers have reported the antibacterial nature of chitosan and chitosan nanoparticles against different species of *Xanthomonas* including *X. garderni* and *X. campestris* (Coqueiro and Di Piero 2011, OH et al 2019, Moon et al 2020, Esyanti et al 2020) which supported our results. However, streptocycline is found to be second best chemical to control the growth of *X. campestris* pv. *mangiferaeindicae*. Tejaswini (2019) also reported streptocycline as the best chemical against this bacterium. The study shows that only *Eucalyptus* leaves extract was able to inhibit the growth of the test bacterium. However, with respect to other species of *Xanthomonas*, the Earlier scientist observed the antibacterial nature of *Eucalyptus* spp. against different species of *Xanthomonas* (Yugander et al 2015, Yemanta et al 2019, Abo Elyousr et al 2020, Sharma 2020).

**Evaluation of different plant extract/ bio-products against *X. campestris* pv. *mangiferaeindicae:*** Among seven plant extracts, cow urine and 10 days old sour butter milk evaluated against the test pathogen, only *Eucalyptus hybrida* leaf extract was able to inhibit the growth of the

**Table 2.** *In vitro* evaluation of different plant extract/ bio-products against *X. campestris* pv. *mangiferaeindicae*

| Plant extracts/bioproducts | Diametric inhibition zone (mm) in different concentration (%) | | | | Overall mean |
|---|---|---|---|---|---|
| | 5 | 10 | 15 | 20 | |
| *Calotropis gigantea* | 0.00 (1.00) | 0.00 (1.00) | 0.00 (1.00) | 0.00 (1.00) | 0.00 (1.00) |
| *Justicia adhatoda* | 0.00 (1.00) | 0.00 (1.00) | 0.00 (1.00) | 0.00 (1.00) | 0.00 (1.00) |
| *Cannabis sativa* | 0.00 (1.00) | 0.00 (1.00) | 0.00 (1.00) | 0.00 (1.00) | 0.00 (1.00) |
| *Murraya koeinigii* | 0.00 (1.00) | 0.00 (1.00) | 0.00 (1.00) | 0.00 (1.00) | 0.00 (1.00) |
| Cow urine | 0.00 (1.00) | 0.00 (1.00) | 0.00 (1.00) | 0.00 (1.00) | 0.00 (1.00) |
| *Lantana camara* | 0.00 (1.00) | 0.00 (1.00) | 0.00 (1.00) | 0.00 (1.00) | 0.00 (1.00) |
| *Azadirachta indica* | 0.00 (1.00) | 0.00 (1.00) | 0.00 (1.00) | 0.00 (1.00) | 0.00 (1.00) |
| *Eucalyptus hybrida* | 6.67 (2.77) | 12.33 (3.56) | 13.67 (3.83) | 16.67 (4.20) | 12.33 (3.61) |
| Sour buttermilk | 0.00 (1.00) | 0.00 (1.00) | 0.00 (1.00) | 0.00 (1.00) | 0.00 (1.00) |
| Overall mean | 0.74 (1.12) | 1.37 (1.23) | 1.52 (1.31) | 1.85 (1.36) | |

| $C.D_{p\geq0.05}$ | S.E.(d) |
|---|---|
| Treatments | 0.02 | 0.01 |
| Concentration | 0.02 | 0.01 |
| Interaction | 0.05 | 0.02 |

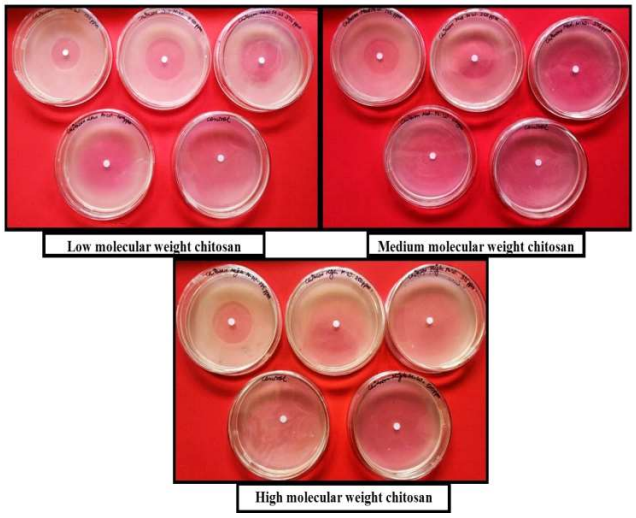


**Plate 1.** Effect of chitosan against *Xanthomonas campestris* pv. *mangiferaindiace*

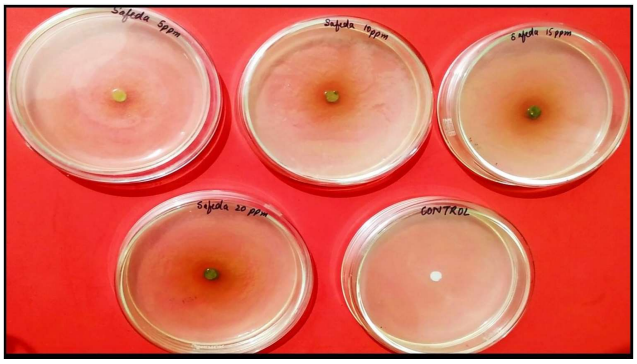


**Plate 2.** Effect of *Eucalyptus hybrida* extract on *Xanthomonas campestris* pv. *mangiferaeindicae*

bacterium (Table 2, Plate 2) with a mean inhibition diametric zone of 12.33 mm. Rest all the plant extracts, cow urine, and 10-day-old sour butter milk failed to inhibit the growth of test bacterium even at their highest concentrations evaluated. In *Eucalyptus hybrida* leaf extract, significantly maximum zone of inhibition (16.67 mm) was recorded at 20 per cent concentration which decreased significantly with reduction in the concentration at each level and was significantly minimum (6.67 mm) at 5 per cent concentration.

## CONCLUSION

Among all various chemicals evaluated *in vitro* against the bacterium by paper disc method, chitosan (high, medium and low molecular weight), streptocycline, Bordeaux mixture, copper hydroxide and copper oxychloride proved to be effective in inhibiting the growth of bacterium as compared to control. Maximum inhibition zone was recorded in case of high molecular weight chitosan at 500 ppm. However, minimum inhibition zone was recorded in case of capstan at 2000 ppm. Among the various plant extracts and natural products evaluated *in vitro* against *X. campestris* pv. *mangiferaeindicae*, only *Eucalyptus hybrida* proved effective in inhibiting the growth of bacterium. Maximum diametric inhibition zone was at 20 per cent concentration and minimum was at 5 per cent concentration of *E. hybrid.*

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