In vitro bio-efficacy of different antibiotics and bio-agents against bacterial canker (*Xanthomonas axonopodis* pv *citri*) of acid lime

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ABSTRACT

Four antibiotics in combination with antibacterial chemicals and two bio-agents viz *Pseudomonas fluorescens* and *Bacillus subtilis*, were evaluated in vitro against *Xanthomonas axonopodis* pv *citri* (Hasse). The results revealed that among the different antibiotics, in combination with antibacterial chemicals, streptocycline (100 ppm) + copper oxychloride (0.25%) showed higher inhibition zone (10.0 mm) as compared to kasugamycin (0.15%) (8.6 mm) and validamycin (0.2%) + copper oxychloride (0.25%) (8.8 mm). In vitro study of bio-agents by dual culture method revealed that *P fluorescence* showed maximum inhibition zone (97.2%) followed by *B subtilis* (92%). In vivo evaluation of antibiotics in combination with antibacterial chemicals and bio-agents revealed that the minimum disease intensity of 19.53 and 19.63 per cent was recorded in streptocycline (100 ppm) + copper oxychloride (0.25%) and streptocycline (100 ppm) + copper hydroxide (0.5%) with 40.41 and 39.46 per cent disease control respectively. Maximum disease intensity was recorded in control (32.50%) with 0.0 per cent disease control followed by *B subtilis* (5 g/l) (27.41%) and *P fluorescence* (5 g/l) (26.43%) with disease control of 16.81 and 19.20 per cent respectively.

Keywords: Bio-efficacy; antibiotics; bio-agents; *Xanthomonas*; acid lime

INTRODUCTION

Citrus is one of the most important fruit crops in India. It occupies an important place in the wealth and economy of India as third largest fruit industry after mango and banana. Citrus belongs to the family Rutaceae and sub-family Aurantiodeae; the role of citrus fruits in providing nutrients and medicinal values has been recognized since ancient times. In India, acid lime [*Citrus aurantifolia* (L) Swingle] is one of the most important citrus fruits grown. It constitutes nearly 20 per cent of the total citrus production in India (Ghosh et al 2010).

Acid lime is grown on a large and commercial scale in different regions such as Akola region in central India, Nellore and Periyakulum regions in southern India and Khera region of western India. Acid lime had good productivity of 12.7 tonnes/ha in India with total production of 13.2 million metric tonnes and was cultivated on 1.034 million ha area during 2019-2020. In Maharashtra state, acid lime is grown on 20.08 thousand hectares with a production of about 201.12 thousand tonnes of fruits annually with productivity of 10.02 tonnes per ha (Anon 2020).

Citrus canker was first reported from Punjab (Luthra and Sattar 1942). The disease caused by the bacterium *Xanthomonas axonopodis* pv *citri* occurs in large areas of the world's citrus growing countries including India. The main symptoms of citrus canker are hyperplasia-type lesions on leaves, fruits and stems. Pathogen lives on cankerous leaves, twigs, branches and fruits during winter (Das 2003).

Citrus leaf miner *(Phallicists citrella)* disseminates the bacteria by carrying it on its body (Sohi and Sandhu 1968). The economic importance of citrus canker can be analyzed from several different points of view. Loss assessment due to it has not been determined clearly, as in the case of diseases of annual crops. Citrus canker has become a permanent major

problem to the citrus growers of the country. Therefore, it becomes very important to have enough further research work to control the disease. There is large number of fungicides and antibiotics available in the market as antibacterial chemicals and their efficacy and stability need to be verified in vitro as well as in vivo, so as to incorporate the effective one in the management package. Considering the importance of the disease, the attempt has been made to evaluate antibiotics in combination with antibacterial chemicals and bio-agents in vitro against *X axonopodis* pv *citri.*

MATERIAL and METHODS

Isolation and purification of *X axonopodis* pv *citri*: The infected leaves of citrus canker were collected from declining orchard, cut into small pieces and crushed in a drop of distilled water. Streak that smeared on NA medium plate with the help of wire loop was incubated for 24 h at 30°C. A loop full culture was streaked on another media plate for pure culture.

In vitro evaluation of antibiotics: The sensitivity of isolate was tested by modified paper disc assay method. The solutions of streptomycin sulphate and bromopol were made with 100 ppm concentrations and kasugamycin with 0.15 per cent, validamycin 0.2 per cent, copper oxychloride 0.25 per cent, copper hydroxide 0.2 per cent and captan 0.25 per cent concentrations. A heavy suspension (72 hours old) of *X axonopodis* pv *citri* multiplied in nutrient broth (20 ml) was mixed with molten (45 to 50°C) nutrient agar medium (1,000 ml) contained in an Erleyenmayer's flask, so as to get the thick growth of bacteria on the medium; 15 to 20 ml of implanted (seeded) medium was poured onto the sterilized Petri plates and allowed to solidify. Eight Petri plates were filled with nutrient agar medium containing bacteria. The medium suspension in plates was allowed to solidify. Filter paper discs (Whatman # 44) measuring 5 mm diameter were soaked separately in the antibiotics formulations and placed onto the surface of the implanted (seeded) nutrient agar medium. Three discs which were previously dipped in the respective formulation solutions in the respective 100 ml flasks, were kept equidistant in each Petri plate. They were considered as three replications (Waksman and Reilly 1945).

The inoculated plates were first incubated at 5°C in refrigerator to allow the diffusion of suspension into the medium and then incubated at 30°C for 72 h. At the end of incubation period, observations were recorded for the production of inhibition zone representing the efficacy of formulations of antibiotics inhibiting the growth of pathogen excluding control. Inhibition zone in each plate was measured in terms of millimeter in diameter and data obtained were analyzed statistically. The paper disc soaked in sterile distilled water served as control.

In vitro evaluation of bioagents: Two bio-control agents viz *Pseudomonas fluorescence* and *Bacillus subtilis* were evaluated for their efficacy against the growth of *X axonopodis* pv *citri* by inhibition zone assay method. The two isolates mentioned were tested using dual culture technique. The 72 h old culture of two bacterial bio-agents and *X axonopodis* pv *citri* were streaked in a single plate containing NA medium with the help of inoculation loop. Two streaks of bio-agents were streaked first and pathogen was steaked at the middle of both steaks of bio-agents. Later, plates were kept for incubation at 30°C for 72 h and finally calculating the inhibition of *X axonopodis* pv *citri* was recorded and data were analyzed statistically as per Donayre and Dalisay (2015).

In vivo evaluation of antibiotics and bio-agents against *X axonopodis* pv *citri*: The greenhouse experiment was conducted at the Department of Plant Pathology and Agricultural Microbiology, Mahatma Phule Krishi Vidyapeeth, Rahuri, Maharashtra to evaluate the efficacy of selected antibiotics in combination with antibacterial chemicals and bio-agents which were found most effective against *X axonopodis* pv *citri* in vitro. Two-year-old healthy seedlings of acid lime grown in pots were used. Observations on acid lime canker disease intensity were recorded on three randomly selected plants for each treatment. The observations were taken at first appearance of the disease ie before spraying. The acid lime canker disease was graded on the basis of disease observed on leaves by applying 0-9 disease rating scale (Mayee and Datar 1986).

RESULTS and DISCUSSION

Results presented in Table 1 indicate that seven formulations of the combination of antibiotics in combination with antibacterial chemicals at various concentrations significantly inhibited the growth of *X axonopodis* pv *citri* over the untreated control. The treatemnts T_1 [Streptocycline (100 ppm) + copper

oxychloride (0.25%)], T_2 [Streptocycline (100 ppm) + copper hydroxide (0.2%)], T_3 [2-bromo-2-nitropropane (100 ppm) + copper oxychloride (0.5%)], T_4 [2-bromo-2-nitropropane (100 ppm) + captan (0.25%)] and T_6 [Validamycin (0.2%) + copper hydroxide (0.2%)] recorded higher inhibition zone of 10.0, 9.6, 9.5, 9.4 and 9.0 mm respectively, which were at par. Lower inhibition zone of 8.6 and 8.8 was obnserved in T_7 [Kasuagmycin (0.15%)] and T_5 [Validamycin (0.2%) + copper oxychloride (0.25%)] respectively, the two being at par with all other treatments except T_1 and T_8 (Control) (0.0 mm). Chakravarti and Rangarajan (1966) reported that streptocycline (100 ppm) was effective in vitro in checking growth of *X campestris* pv *citri.* Similarly, Yenjerappa et al (2004) reported superior efficacy of streptocycline (0.05%) in combination with copper oxychloride (2,000 ppm) in controlling the bacterial blight of pomegranate in vitro.

The efficacy of bio-agents tested against *X axonopodis* pv *citri* (Fig 1) revealed that the test bio-agents significantly inhibited bacterial growth of *X axonopodis* pv *citri* over untreated control. The *P fluorescence* (5 g/l) resulted in the highest inhibition (97.2%), whereas, *B subtilis* (5 g/l) recorded 92 per cent inhibition. The results are in conformity with the work of Raju et al (2013) who evaluated biocontrol agents viz *P fluorescens* and *B subtilis* for their efficacy against the growth of *X axonopodis* pv *punicae* by dual culture method. Studies indicated that among the four antagonists tested, *P fluorescens* was found to be significantly superior in inhibiting the growth of bacterium followed by *B subtilis*.

In vivo efficacy of antibiotics and bio-agents against *X axonopodis* pv *citri*: The data presented in Table 2 reveal that the antibiotics in combination

Table 1. In vitro efficacy of antibiotics in combination with antibacterial chemicals against bacterial canker of acid lime

Treatment	Mean diameter of inhibition zone (mm)	Inhibition zone (%)
T_1: Streptocycline (100 ppm) + copper oxychloride (0.25%)	10.0	11.11
T_2: Streptocycline (100 ppm) + copper hydroxide (0.2%)	9.6	10.6
T_3: 2-bromo-2-nitropropane (100 ppm) + copper oxychloride (0.5%)	9.5	10.5
T_4: 2-bromo-2-nitropropane (100 ppm) + captan (0.25%)	9.4	10.4
T_5: Validamycin (0.2%) + copper oxychloride (0.25%)	8.8	9.8
T_6: Validamycin (0.2%) + copper hydroxide (0.2%)	9.0	10,0
T_7: Kasuagmycin (0.15%)	8.6	9.6
T_8: Control	0.0	0.0
SEm(±)	0.35	-
$CD_{0.01}$	1.07	-

Table 2. Canker disease intensity and control influenced by different antibiotics and bio-agents in acid lime

Treatment	Disease intensity (%)	Disease control (%)
T_1: Streptocycline (100 ppm) + copper oxychloride (0.25%)	19.53 (26.23)	40.71
T_2: Streptocycline (100 ppm) + copper hydroxide (0.2%)	19.63 (26.30)	39.46
T_3: 2-bromo-2-nitropropane (100 ppm) + copper oxychloride (0.5%)	20.92 (27.22)	36.15
T_4: 2-bromo-2-nitropropane (100 ppm) + captan (0.25%)	21.95 (27.94)	32.95
T_5: Validamycin (0.2%) + copper oxychloride (0.25%)	24.0 (29.33)	25.75
T_6: Validamycin (0.2%) + copper hydroxide (0.2%)	24.9 (29.93)	24.95
T_7: Kasuagmycin (0.15%)	25.23 (30.15)	19.70
T_8: *Pseudomonas fluorescence* (5 g/l)	26.43 (30.94)	19.20
T_9: *Bacillus subtilis* (5 g/l)	27.41 (31.57)	16.81
T_{10}: Control	32.50 (34.76)	0.0
SEm(±)	0.23	-
$CD_{0.05}$	0.63	-

Figures in parentheses are arc sine transformed values

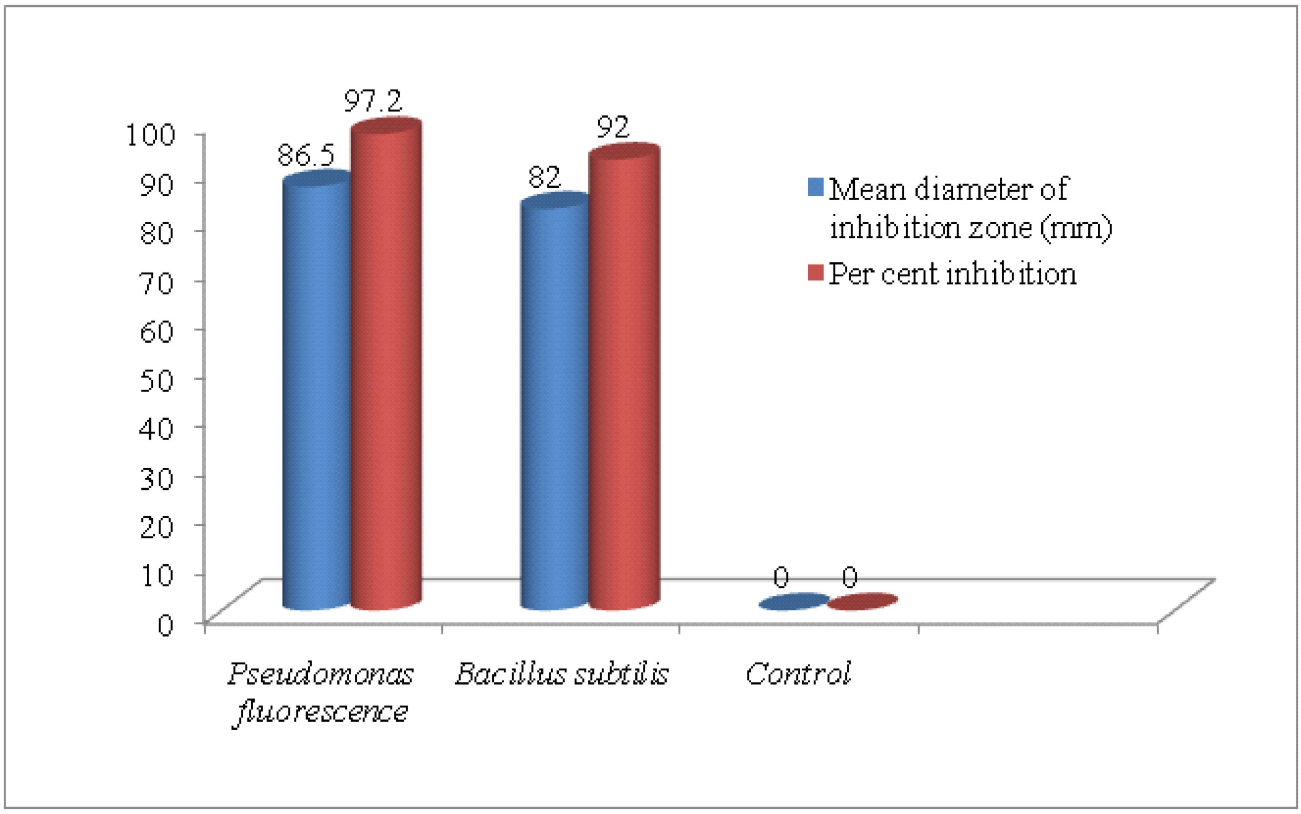

Fig 1. In vitro efficacy of bio-agents by dual culture method

with antibacterial chemicals and bio-agents were found effective and significantly reduced the canker disease intensity over untreated control. The treatments T_1 and T_2 recorded the minimum disease intensity of 19.53 and 19.63 per cent respectively, which were par, as compared to all other treatments. The highest disease control was recorded in T_1 (40.71%) followed by T_2 (39.46%). Maximum disease intensity was recorded in T_{10} (Control) (32.50%) with 0.0 per cent disease control followed by T_9 [*Bacillus subtilis* (5 g/l)] (27.41%) and T_8 [*P fluorescence* (5 g/l)] (26.43%), the two being at par. T_9 and T_8 resulted in deisease control of 16.81 and 19.20 per cent respectively.

Similar results were reported by Kale et al (1994) who observed that the spraying of streptocycline + copper oxychloride gave better control of citrus canker. Patel and Desai (1970) reported that four sprays of streptocycline + copper oxychloride were effective in checking canker in acid lime.

CONCLUSION

It can be concluded that the *P fluorescence* recorded maximum inhibition zone (97.2%) followed by *B subtilis* (92%). In vivo evaluation of antibiotics in combination with antibacterial chemicals and bio-agents revealed that the minimum disease intensity of 19.53 and 19.63 per cent was recorded due to streptocycline (100 ppm) + copper oxychloride (0.25%) with 40.71 per cent disease control and streptocycline (100 ppm) + copper hydroxide (0.5%) (19.63%) with 39.46 per cent disease control.

REFERENCES

Anonymous 2020. National horticulture board database. National Horticulture Board, Ministry of Agriculture and Farmers' Welfare, Government of India.

Chakravarti BP and Rangrajan M 1966. Streptocycline, an effective antibiotic against bacterial plant pathogens. Hindustan Antibiotics Bulletin **8(4):** 209-211.

Das AK 2003. Citrus canker – a review. Journal of Applied Horticulture **5(1):** 52-60.

Donayre DKM and Dalisay TU 2015. Potential of endophytic fungi of barnyard grass weed for biological control of the rice sheath blight pathogen, *Rhizoctonia solani* Kuhn. International Journal of Philippine Science and Technology **8(2):** 48-51.

Ghosh DK, Das AK, Singh S, Singh SJ and Ahlawat YS 2010. Association of phytoplasma with witches' broom, a new disease of acid lime (*Citrus aurantifolia*). Current Science **77(1):** 174-177.

Kale KB, Kolte SO and Peshney NL 1994. Economics of chemical control of citrus canker caused by *Xanthomonas campestris* pv *citri* under field conditions. Indian Phytopathology **47(3):** 253-255.

Luthra JC and Sattar A 1942. Citrus canker and its control in Punjab. Punjab Fruit Journal **6(1):** 179-182.

Mayee CD and Datar VV 1986. Phytopathometry. Marathwada Agricultural University, Parbhani, Maharashtra, India, 146p.

Patel RS and Desai MV 1970. Control of citrus canker. Indian Journal of Horticulture **27:** 93-98.

Raju J, Benagai VI, Jayalakshmi K, Angadi SG, Basha H and Giri MS 2013. In vitro evaluation of bio-prospectus for bacterial blight of pomegranate. Bioinfolet **10(2B):** 710-713.

Sohi GS and Sandhu MS 1968. Relationship between citrus leaf miner (*Phyllocnistis citrella* Stainton) injury and citrus canker [*Xanthomonas citri* (Hasse) Downson] incidence on citrus leaves. Journal of Research, PAU **5(1):** 66-69.

Waksman SA and Reilly HC 1945. Agar-streak method for assaying antibiotic substances. Industrial and Engineering Chemistry Analytical Edition **17(9):** 556-558.

Yenjerappa ST, Ravikumar MR, Jawadagi RS and Khan NA 2004. In vitro and in vivo efficacy of bactericides against bacterial blight of pomegranate. Paper presented in National Symposium on Crop Surveillance: Disease Forecasting and Management, 19-21 February 2004, New Delhi, India.