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In-vitro evaluation of different agrochemicals and medicinal plant extracts against *Xanthomonas citri* PV. *Citri* causing citrus canker

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Abstract
Of all the agricultural pests and diseases that feared citrus crops, citrus canker is one of the most devastating. The disease, caused by the bacterium *Xanthomonas axonopodis* pv. *citri*, distributed worldwide including India. The initial symptoms appeared on leaves in the form of lesions as dark green and later become thickened brown and corky which become raised and blister-like, growing into white or yellow spongy pustules. In present study, bacteria was isolated on Nutrient agar medium from infected leaves and fruits collected from Horticultural farm, College of Horticulture, AAU, Anand which showed yellow, circular and mucoid colony. Purification of bacteria was done by using streak plate method. *In-vitro* efficacy of different agrochemicals and medicinal plant extracts were assessed using Agar-well diffusion method. Among ten tested agrochemicals, streptomycin sulphate 90% + tetracycline hydrochloride 10% SP mixed with copper oxychloride 50% WP showed highest zone of inhibition with all three concentration (100, 200 and 300 ppm) 20.28 mm, 22.62 mm and 26.08 mm with percent inhibition of 22.54, 25.13 and 28.97. Among medicinal plant extracts, highest zone of inhibition (7.91 and 12.20 mm) was recorded with treatment Neem with percent inhibition of 8.78 and 13.55 percent.

Keywords: *Xanthomonas citr*i pv. *citri*, *Xac*, Nutrient agar media, Nutrient broth

Introduction
The genus Citrus is one of the most important group of fruit crops worldwide, belongs to the family *Rutaceae* comprising 140 genera and 1300 species distributed throughout the world (Saunt, 1990, Savita *et al.*, 2012) [23, 24]. It is a long-lived perennial crop and is grown in more than 100 countries across the world. Among the various fungal, bacterial and viral diseases, few diseases in any citrus growing area cause significant damage and require due attention for their effective management. Among which citrus canker caused by *Xanthomonas citri* pv. *citri* (*Xac*) is the most damaging one. Citrus canker signifies as most devastating threat to citrus and cause severe losses in the world (Das, 2003) [4]. The disease is endemic in India, Japan and other South-East Asian countries, from where it has spread to all other citrus producing continents. In India, citrus canker was first reported from Punjab (Luthra and Sattar, 1942) [15]. Then its occurrence was further recorded in Assam (Chowdhury, 1951) [3], Tamil Nadu (Ramakrishnan, 1954) [22], Andhra Pradesh (Govinda Rao, 1954) [10], Karnataka (Venkatakrishnaiah, 1957, Aiyappa, 1958) [26, 1], Rajasthan (Prasad, 1959) [20], Madhya Pradesh (Parsai, 1959) [18], Uttar Pradesh (Nirvan, 1960) [17] and several others have reported the incidence of canker from different state. The causative pathogenic bacterium was first isolated by Hasse in 1915 from diseased samples of Florida, Texas and Mississippi and he named it *Pseudomonas citri* following its characterization and pathogenicity in citrus (Hasse, 1915) [12]. Subsequently it was placed under different genera by different workers and in 1939 it was named as *Xanthomonas citri* (Doidge, 1916, Dowson, 1939) [7, 8]. *Xanthomonas citri* pv. *citri* of *Xanthomonadaceae* family is a straight rod-shaped measuring 1.5- 2.0 × 0.5-0.75 µm, mono-flagellum, gram negative bacteria which give yellowish colonial growth due to production of xanthomonadin pigment (Pitino *et al.*, 2015) [19] Das (2003) [4]. The citrus canker produces typical necrotic lesions on stem, leaves and fruits. The disease causes leaf fall, drying of twigs and premature fruit drop which leads to low quality fruits and yield losses (Gottawald *et al.*, 2002, Graham *et al.*, 2004) [9, 11]. One of the key strategies being used in management of disease is use of resistant verities.

Use of such disease resistant verities is of great help in disease management and saving losses (Deng *et al.*, 2009) [6] but this comes with additional constraints including a long-term durational span required for cross breeding and checking of each generation separately, attaining of additional traits which are of no good in disease management and probably exerts fitness penalty reducing plant yields. Over the time, canker disease has been tried to be managed by use of various synthetic chemicals. For management of bacterial diseases various bactericides having copper as an active ingredient has been used.

There are number of programmers are working on the management of these disease but the complete solution to the problem is yet to be found except the eradication. The antibiotics with bactericidal nature are the most commonly used for the management of these disease. Keeping in view the great economic importance of citrus canker disease, this experiment was taken to test the effect of antibiotics and medicinal plant extracts against the *Xanthomonas citri* pv. *citri* causing citrus canker disease.

Materials and Methods

Collection of samples

The infected plant parts like leaves, twigs and fruits showing typical symptoms of citrus canker were collected from the Horticulture farm, College of Horticulture, AAU, Anand in kharif, 2020 and brought to the laboratory for microscopic examination followed by isolation.

Isolation and purification of bacteria

Small pieces of canker infected tissue like leaves, twigs and fruit pericarp were cut and surface sterilized using 1% sodium hypochlorite disinfectant. The surface sterilized tissues were kept in a screw cap tube having 3 ml of sterilized water for 6 hrs at room temperature. Suspension was streaked on Nutrient Agar medium plate with the help of wire loop and incubated for 24 hrs at 30 ℃. A single bacterial colony was transferred on another media plate for pure culture.

In-vitro evaluation of different agrochemicals

Susceptibility of *Xanthomonas citri* pv. *citri* to different agrochemicals was determined *in-vitro* by employing Agar-well diffusion method. The bacterium *Xanthomonas citri* pv. *citri* was multiplied by inoculating the loop full culture in 250 ml conical flask containing 100 ml of Nutrient broth medium and incubated at 28±2 ℃ for 72 hours. The 20 ml bacterial suspension was added to molten and cooled 1000 ml nutrient agar medium at temperature 28±2 ℃. The seeded medium was thoroughly mixed and poured into the sterilized Petri plates and allowed to solidify. A well of 5 mm diameter was made by sterilized cork-borer on the four corner of the plate and each well was poured with 50 µl of various agrochemicals at three concentrations *i.e.*, 100, 200 and 300 ppm. The diameters of zone of inhibition produced by different agrochemicals were measured in millimeters (mm) scale. The percent growth inhibition over control was calculated using the formula given by Vincent (1947) [27]. The results obtained were analyzed statistically.

$$I = DC - \frac{DT}{DC} \times 100$$

Where,

I = Percent inhibition zone

DC = Colony diameter in control plate (mm)

DT= Colony diameter in treated plate (mm)

In-vitro evaluation of medicinal plant extracts

Sensitivity of *Xanthomonas citri* pv. *citri* to different nineteen medicinal plant extracts at 5 and 10 percent concentration were tested *in-vitro* using Agar-well diffusion method. Before preparation of extracts, each medicinal plants were dipped in 1 percent sodium hypochlorite solution for 1 minute. The extracts were prepared by grinding 100 g of washed leaf/stem of different species in 100 ml distilled water with mixture-cum grinder. All extracts were filtered through muslin cloth and centrifuged at 5000 rpm for 15 min. The final clear filtrate obtained was treated as 100 percent concentration of these extracts. The percent growth inhibition over control was calculated using the formula as per given in agrochemicals.

Results and Discussion

Isolation of bacteria from diseased specimens

Bacterial pathogen *Xanthomonas citri* pv. *citri* was isolated on Nutrient agar medium from infected leaves, twigs and fruits collected from Horticultural farm, College of Horticulture, AAU, Anand and incubated at 28 ℃. After 24 hrs of incubation, yellow, smooth, convex and circular colonies were observed which became somewhat irregular after 72 hrs due to viscous fluid secreted by the bacteria. The bacteria was purified using streak plate method.

Sun *et al.* (2004) [25], Islam *et al.* (2014) [13] and Daungfu *et al.* (2019) [5] isolated bacteria from collected diseased samples on NA media and observed yellow, smooth, convex and circular colonies of bacteria.

In-vitro evaluation of agrochemicals

Ten agrochemicals at different concentration *viz.*, 100, 200 and 300 ppm were used *in-vitro* for testing their efficacy against citrus canker causing bacteria *Xanthomonas citri* pv. *citri* using Agar-well diffusion technique. The result is presented in Table 1. All the antibiotics significantly reduced the growth of *X. citri* pv. *citri* as compared to control. The highest zone of inhibition (20.28, 22.62 and 26.08 mm) and percent inhibition (22.54, 25.13 and 28.97%) were recorded in streptomycin sulphate 90% + tetracycline hydrochloride 10% SP mixed with copper oxychloride 50% WP (T_5) with all three concentration (100, 200 and 300 ppm), respectively followed by streptomycin sulphate 90% + tetracycline hydrochloride 10% SP mixed with bordeaux mixture 1% and streptomycin sulphate 90% + tetracycline hydrochloride 10% SP. The lowest zone of inhibition was recorded in kasugamycin 3% SL (T_3) followed by kasugamycin 5% + copper oxychloride 45% WP with all three concentrations.

The same results as present investigation was obtained by Raju *et al.* (2012) [21] who revealed that highest zone of inhibition was observed under streptocycline + COC of 3.3 cm followed by streptocycline (2.80 cm) and COC (2.65 cm).

In-vitro evaluation of different medicinal plant extracts

In the present investigation, 19 medicinal plant extracts were screened using Agar-well diffusion technique *in-vitro* so as to

know their inhibitory effect on the growth of *X. citri* pv. *citri* (Table 2). The highest zone of inhibitions (7.91 and 12.20 mm) and percent inhibition (8.78 and 13.55%) were recorded in Neem followed by Ardusi with 6.32 and 9.31 mm of zone of inhibition and 7.02 and 10.34 percent inhibition at 5 and 10 percent concentration, respectively. Other seventeen treatments were found non-significant to reduce the growth of *X. citri* pv. *citri*.

The results of the present investigation are in confirmation with the results obtained by Negi and Kumar (2015) [16] who observed formation of highest zone of inhibition *i.e.*, 1.73 cm with treatment Neem followed by Garlic (1.67 cm) at 20 percent concentration. Atiq *et al.* (2018) [2] also observed highest zone of inhibition with treatment Neem among the tested plant extracts. Kharat *et al.* (2020) [14] found Neem leaf extract as a most effective in alcoholic extracts by developing 13.45, 11.70, 10.12 and 8.2 mm of inhibition zone at 20, 15, 10 and 5 percent, respectively.

Table 1: Evaluation of different agrochemicals against *X. citri* pv. *citri in-vitro*

Sr. No.	Treatments	Zone of inhibition (mm)			Percent inhibition		
		100ppm	200ppm	300ppm	100ppm	200ppm	300ppm
1	Streptomycin sulphate 22.4% SP	11.95^{d}	13.25^{e}	16.87^{d}	13.28	14.72	18.74
2	Streptomycin sulphate 90%+ Tetracycline hydrochloride 10% SP	15.67^{c}	17.52^{c}	21.25^{c}	17.41	19.47	23.50
3	Kasugamycin 3% SL	4.67^{h}	7.27^{h}	8.14^{h}	5.19	8.07	9.04
4	Validamycin 3% L	7.10^{g}	9.29^{g}	10.39^{g}	7.88	10.32	11.54
5	Streptomycin sulphate 90%+ Tetracycline hydrochloride 10% SP & Copper oxychloride 50% WP (Mixed)	20.28^{a}	22.62^{a}	26.08^{a}	22.54	25.13	28.97
6	Streptomycin sulphate 90%+ Tetracycline hydrochloride 10% SP & Bordeaux mixture 1% (Mixed)	17.35^{b}	20.80^{b}	24.26^{b}	19.27	23.11	26.95
7	Copper hydroxide 53.5 DF	10.19^{e}	12.13^{f}	15.04^{e}	11.32	13.48	16.71
8	Copper oxychloride 50% WP	12.54^{d}	15.27^{d}	16.17^{de}	13.94	16.96	17.96
9	Kasugamycin 5% + Copper oxychloride 45% WP	9.12^{f}	9.44^{g}	9.93^{g}	10.13	10.48	11.03
10	Hexaconazole 5% + Validamycin 2.5% SC	12.16^{d}	13.05^{ef}	13.33^{f}	13.51	14.50	14.81
11	Control	0.00^{i}	0.00^{i}	0.00^{i}	0.00	0.00	0.00
S.Em. ±		0.274	0.306	0.379	-	-	-
C.D. at 5%		0.803	0.896	1.113	-	-	-
C.V. (%)		4.31	4.14	4.48	-	-	-

Note: Treatments means with the letter/letters in common are not significant by Duncan's New Multiple Range Test at 5 percent level of significance

Table 2: Evaluation of different medicinal plant extracts against *X. citri* pv. *citri in-vitro*

Tr. No.	Treatments	Zone of inhibition (mm)		Percent inhibition	
		5%	10%	5%	10%
1	Neem - *Azadirachta indica* A.Juss.	7.91^{a}	12.20^{a}	8.78	13.55
2	Ardusi / Malabar nut - *Justicia adhatoda* L.	6.32^{b}	9.31^{b}	7.02	10.34
3	Kalmegh / Green chiretta - *Andrographis paniculata* (Burm. f.) Nees	0.00^{d}	0.00^{h}	0.00	0.00
4	Brahmi / Indian pennywort - *Centella asiatica* (L.) Urb.	0.00^{d}	5.31^{g}	0.00	5.90
5	Barmasi / Periwinkle - *Catharanthus roseus* (L.) G. Don	0.00^{d}	5.87^{f}	0.00	6.52
6	Kadvoindrajav - *Holarrhena pubescens* Wall.	0.00^{d}	7.98^{c}	0.00	8.86
7	Asalio / Garden cress - *Lepidium sativum* L.	0.00^{d}	0.00^{h}	0.00	0.00
8	Viklo / Vikantata - *Gymnosporia emarginata* (Willd.) Thwaites	0.00^{d}	0.00^{h}	0.00	0.00
9	Vardharo / Morning-glories - *Argyreia nervosa* (Burm. f.) Bojer	0.00^{d}	0.00^{h}	0.00	0.00
10	Panphuti / Miracle leaf - *Bryophyllum pinnatum* (Lam.) Oken	0.00^{d}	0.00^{h}	0.00	0.00
11	Chanothi / Jequirity bean - *Abrus precatorius* L.	0.00^{d}	0.00^{h}	0.00	0.00
12	Kuvadio / Sickle Senna - *Cassia tora* L.	0.00^{d}	0.00^{h}	0.00	0.00
13	Mindhiaaval / Senna - *Senna alexandrina* Mill.	0.00^{d}	7.31^{d}	0.00	8.12
14	Nagol / Five-leaved chaste tree - *Vitex negundo* L.	5.92^{c}	9.57^{b}	6.58	10.64
15	Giloy / Guduchi - *Tinospora cordifolia* (Willd.) Miers	0.00^{d}	0.00^{h}	0.00	0.00
16	Parijat / Night-flowering jasmine - *Nyctanthes arbor-tristis* L.	0.00^{d}	0.00^{h}	0.00	0.00
17	Nagarvel - *Piper betle* L.	0.00^{d}	6.41^{e}	0.00	7.12
18	Ashwagandha / Indian ginseng - *Withania somnifera* (L.) Dunal	0.00^{d}	6.04^{f}	0.00	6.71
19	Paneer dodi / Punir bundh - *Withania coagulans* (Stocks) Dunal	0.00^{d}	8.26^{c}	0.00	9.18
20	Control	0.00^{d}	0.00^{h}	0.00	0.00
S.Em. ±		0.0219	0.098	-	-
C.D. at 5%		0.0627	0.279	-	-
C.V. (%)		3.66	4.32	-	-

Note: Treatments means with the letter/letters in common are not significant by Duncan's New Multiple Range Test at 5% level of significance

Conclusions

The bacteria causing citrus canker was isolated from affected leaves and fruits collected from Horticultural farm, College of Horticulture, AAU, Anand on Nutrient agar media which produce smooth, yellow convex, mucoid and circular colony after 24 hrs of incubation. Among the ten agrochemicals checked, highest zone of inhibition of 20.28, 22.62 and 26.08 mm was observed with treatment streptomycin sulphate 90% + tetracycline hydrochloride 10% SP mixed with copper oxychloride 50% WP with percent inhibition of 22.54, 25.13

and 28.97 at 100, 200 and 300 ppm concentration, respectively followed by streptomycin sulphate 90% + tetracycline hydrochloride 10% SP mixed with bordeaux mixture 1% (mixed). From all evaluated medicinal plant extracts, highest zone of inhibition was recorded with treatment Neem 7.91 mm and 12.20 mm with percent inhibition of 8.78 and 13.55 at 5 and 10 percent concentration, respectively followed by Ardusi.

Conflict of interest: The author declare that they have no conflict of interest.

Statement on human and animal rights: This article does not contain any studies with human participants or animals performed by any of the authors.

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References

1. Aiyappa KM. Citrus canker - *Xanthomonas citri* (Hasse) Dowson. Mysore Journal of Agricultural Science. 1958;13:164-167.
2. Atiq M, Aslam Khan M, Talib Sahi S, Ahmad R, Younas M, Shafiq M, *et al*. Appraisal of plant extracts and streptomycin sulphate against citrus canker disease. Archives of Phytopathology and Plant Protection. 2018;51(15-16):824-833.
3. Chowdhury S. Citrus canker in Assam. Plant Protection Bulletin. 1951;3:78-79.
4. Das AK. Citrus canker-A review. Journal of Applied Horticulture. 2003;5(1):52-60.
5. Daungfu O, Youpensuk S, Lumyong S. Endophytic bacteria isolated from citrus plants for biological control of citrus canker in lime plants. Tropical Life Sciences Research. 2019;30(1):73.
6. Deng ZN, Xu L, Li DZ, Long GY, Liu LP, Fang F, *et al*. Screening citrus genotypes for resistance to canker disease (*Xanthomonas axonopodis* pv. *citri*). Plant Breeding. 2009;129:341-345.
7. Doidge EM. Citrus canker in South Africa. South African Fruit Grower. 1916;3:265-268.
8. Dowson WJ. On the systematic position and generic names of the Gram-negative bacterial plant pathogens. On the systematic position and generic names of the Gram-negative bacterial plant pathogens; c1939. p. 9-13.
9. Gottwald TR, Sun X, Riley T, Graham JH, Ferrandino F, Taylor EL, *et al*. Geo-Referenced Spatiotemporal Analysis of the Urban Citrus Canker Epidemic in Florida. Phytopathology. 2002;92:361-377.
10. Govinda Rao P. Citrus diseases and their control in Andhra State. Andhra Agricultural Journal. 1954;1:187-192.
11. Graham JH, Gottwald TR, Cubero J, Achor DS. *Xanthomonas axonopodis* pv. *citri*: Factors affecting successful eradication of citrus canker. Molecular Plant Pathology. 2004;5(1):1-15.
12. Hasse CH. *Pseudomonas citri,* the cause of citrus canker. Journal of Agricultural Research. 1915;4(1):97-100.
13. Islam MA, Mazumdar RM, Islam S, Alam MJ, Urmee SA. Isolation, identification and *in-vitro* antibiotic sensitivity pattern of citrus canker causing organism *Xanthomonas axonopodis*. Advancements in Life Sciences. 2014;1(4):215-222.
14. Kharat VM, Bramhankar SB, Thakur KD, Isokar SS, Pillai T, Dinkwar GT, *et al*. Management of citrus canker bacterium by using botanicals and chemicals. International Journal of Chemical Studies. 2020;8(2):1878-1882.
15. Luthra JC, Sattar A. Citrus canker and its control in Punjab. Punjab Fruit Journal. 1942;6(1):179-182.
16. Negi A, Kumar P. Antibacterial effect of plant extracts and antibiotics on *Xanthomonas axonopodis* pv. *citri in vitro*. Trends in Biosciences. 2015;8(9):2374- 2376.
17. Nirvan RS. Effect of antibiotic sprays on citrus canker. Advance in Horticulture. 1960;4:155-160.
18. Parsai PS. Citrus canker., Procurement Seminar on Diseases of Horticultural Plants Simla; c1959. p. 91-95.
19. Pitino M, Armstrong CM, Duan Y. Rapid screening for citrus canker resistance employing pathogen associated molecular pattern triggered immunity responses. Horticulture Research. 2015;2(1):1-8.
20. Prasad N. Citrus canker. Procurement Seminar on Disease of Horticultural Plants, Simla; c1959. p. 87-88.
21. Raju J, Benagi VI, Jayalakshmi K, Nargund VB, Sonavane PS. *Invitro* evaluation of chemicals, botanicals and bioagents against the bacterial blight of pomegranate caused by *Xanthomonas axonopodis* pv. *punicae*. International Journal of Plant Protection. 2012;5:315-318.
22. Ramakrishnan TS. Common diseases of citrus in Madras state. Government of Madras publication; c1954. p. 130-134.
23. Saunt J. Citrus varieties of the world. Sinclair International Ltd; c1990. p. 126.
24. Savita, Virk GS, Nagpal A. Citrus diseases caused by Phytophthora species. In: GERF Bulletin of Biosciences. 2012;3(1):18-27.
25. Sun X, Stall RE, Jones JB, Cubero J, Gottwald TR, Graham JH, *et al*. Detection and characterization of a new strain of citrus canker bacteria from Key/Mexican lime and alemow in South Florida. Plant disease. 2004;88(11):1179-1188.
26. Venkatakrishnaiah NS. Canker disease of sour lime and its control. Journal of Mysore Horticultural Science. 1957;2(2, 3):40-44.
27. Vincent JM. Distortion of fungal hyphae in the presence of certain inhibitors. Nature. 1947;159(1):850.