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Assessment of allopathic and homeopathic treatments for inhibiting *Xanthomonas citri* pv. *citri* in citrus canker management

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Abstract

Citrus canker caused by *Xanthomonas citri* pv. *citri* is emerging as serious threat to worldwide citrus production and its control is becoming important. *In vitro* evaluation of various allopathic antibiotics showed the highest zone of inhibition was recorded in streptomycin sulphate 90% + tetracycline hydrochloride 10% SP with all three concentrations i.e., 50, 100 and 150 ppm with 18.67, 20.67 and 22.33 mm, respectively. The next best treatment was Levofloxacin with all three concentrations with 15.67, 17.67 and 20.33-mm inhibition zone. Among the various homeopathic medicines, the highest zone of inhibitions was recorded in Lachesis with three concentrations, 5, 10, and 15 percent with 3.65, 4.28 and 4.62-mm inhibition zone followed by phosphorus with 2.31, 2.31 and 4.00 mm at 5, 10 and 15 percent concentration, respectively.

Keywords: Citrus canker, management, allopathic antibiotics, homeo medicines

Introduction

Citrus forms the backbone of the agriculture industry in many countries (Ismail and Zhang, 2004) [7]. It is most important evergreen fruit crop in the world. It is originated in South East Asia and spread during the middle Ages, later become established in all continents. It occupies a place of considerable importance in the fruit economy of the country. It comprises the third largest fruit industry after mango and banana and occupies about 7.5% of land under fruits (Sidana *et al.*, 2013) [18]. Citrus species grown in the world suffers from more than 100 diseases Klotz (1973) [8]. It is one of the most devastating diseases throughout the world that affects many kinds of commercial citrus varieties. Citrus canker has an extensive history in Florida. The pandemics that occurred in Mexico in 1981 and Florida in 1984 were non-identical to the Asiatic strain of canker (Schubert and Miller, 2004; Das, A. 2003) [16, 1]. A large-scale epidemic was also found in urban Miami, Florida in 1995. In some of these locations, efforts were made to remove the bacteria causing disease, but not all efforts were successful. It was rediscovered in 1997, and the efforts to remove this disease began again Graham (2004) [3]. In India, citrus canker was first reported by from Punjab (Luthra and Sattar, 1942) [11] and now prevalent in Tamil Nadu, Andhra Pradesh, Rajasthan, Karnataka, Madhya Pradesh, Assam, Himachal Pradesh, and Uttar Pradesh Nirvan (1980) [15]. This disease is presently occurring in thirty countries in Asia, the Pacific and Indian ocean islands, South America, and the South eastern USA Das (2003) [1]. Symptoms are characterized mainly by the formation of erumpent, corky, and raised pustules on the surface of leaves, fruits, and twigs, which serve as sources of bacterial inoculums. These early symptoms show as early as 4–7 days after the entry of bacterium into the plant tissues under ideal conditions, which include the presence of water film and temperatures in the range of 20–30 °C (Koizumi *et al.*, 1985) [9]. If the conditions are not ideal, the symptoms take longer than expected, i.e., more than 60 days Loucks (1934) [10]. Defoliation and fruit drop are also observed as plant responses to the infection. *X. axonopodis* pv. *citri* is a rod-shaped Gram-negative bacterium with single polar flagellum and it requires maximum temperature of 35 to 39 °C and obligatorily aerobic conditions for its growth Mehrotra (1980) [12]. They produce typical yellow colour colonies having a carotenoid pigment is indicative of genus *Xanthomonas* (Starr, M. P and Stephens, 1964) [19].

There are five major pathovars belonging to *Xanthomonas citri* that are responsible for causing diseases in citrus, i.e., citri, aurantifolii (Pathotype B, C, D) and citrumelo (also referred to as type E), and they also have many bacterial strains (Vauterin *et al.*, 1991; Vauterin *et al.*, 1995; Sun *et al.*, 2004) [22, 21, 20]. These five pathovars were initially studied as causal agents of citrus canker A, B, C, D, and E, respectively, but detailed studies have highlighted a controversy about the causative agents of citrus canker D and E. Problems of resistance for the recommended bactericides in the market against streptomycin have increased. The severity and significance of damage due to canker have necessitated the development of strategies to manage the disease in citrus. It has become mandatory to substitute the conventional method of disease management. Hence, the study was undertaken to carry out investigation on the management of pathogen using allopathic antibiotics and homeopathic medicines.

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, B. A. College of Agriculture, AAU, Anand in *kharif*, 2022 and brought to the laboratory for further examination.

Isolation and purification of Pathogen

Small pieces of canker infected tissue along with healthy tissue were disinfected in 1% sodium hypochlorite disinfectant for 1 minute and washed thoroughly with a sterile distilled water, dried on sterile blotter paper. The surface sterilized tissues were transferred into 3 ml of sterilized water and incubated for 6 hrs at room temperature. Then, loopful amount of suspension streaked on NA plates. The inoculated plates were incubated at 28 ± 1 °C. A single purified bacterial colony was isolated and purified again on Nutrient agar medium and then it was kept in refrigerator at 4 °C for further use.

In vitro evaluation of allopathic antibiotics

In the current study commonly available antibiotics viz., ampicillin, gentamicin, kanamycin sulphate, benzylpenicillin, tetracycline, vancomycin hydrochloride, levofloxacin, ciprofloxacin, clarithromycin, azithromycin, cefotaxime, Streptomycin sulphate (90%) + Tetracycline hypochloride (10%) were evaluated against the *Xanthomonas citri* pv. *citri* at different concentrations viz., 50, 100 and 150 ppm were made by using the stock solution to examine the effectiveness of antibiotics against the colonial growth and inhibition zone of the pathogen.

Disk diffusion technique

Disk diffusion method was used to determine the effectiveness of the antibiotics against the *Xanthomonas citri* pv. *citri*. Three days old bacterial culture of *X. citri* pv. *citri* was maintained in nutrient broth. Molten nutrient agar was inoculated with bacterial culture maintained in nutrient broth @ 1 ml/100 ml of nutrient agar. Nutrient agar was poured into the sterilized Petri plates and allowed to solidify. Then, filter paper was made into 6 mm paper discs and were dipped in the antibiotic solution, kept on the agar surface, and labelled accordingly. Three repetitions were kept for each concentration of respective treatments. The

discs which were dipped in the distilled water was kept as control. The observation on inhibition zone (mm) of test bacteria was recorded after 48 hrs of incubation.

In vitro evaluation of Homeopathic medicines

In the current study commonly available Homeopathic medicines viz., Argentum nitricum 30 CH, Calcium carbonicum 30 CH, Carbo animalis 30 CH, equisetum 30 CH, Lachesis 30 CH, Phosphorus 30 CH, Zincum sulfuricum 30 CH were evaluated against the *Xanthomonas citri* pv. *citri* at different concentrations viz., 5, 10 and 15 percent were made to examine the effectiveness of treatments against the colonial growth and inhibition zone of the pathogen.

Agar well diffusion method

Agar well diffusion method was used to determine the effectiveness of homeopathic medicines against *X. citri* pv. *citri*. Three days old bacterial culture of *X. citri* pv. *citri* was maintained in nutrient broth. Molten nutrient agar was inoculated with bacterial culture-maintained in nutrient broth @ 1ml/100 ml of nutrient agar. Nutrient agar was poured into the sterilized Petri plates and allowed to solidify. A well (5 mm in diameter) was made by piercing the nutrient agar with a sterilized cork borer on the corner of the plate in four directions by leaving 1 cm from the periphery of the plates. Each well was poured with 50 µl of treatments at different concentrations. Three repetitions were kept for each concentration of respective treatments. The growth of test bacteria on non treated NA media was served as a control. The observation on inhibition zone (mm) of test bacteria was recorded after 48 hrs of incubation.

Results and Discussion

Isolation and purification of pathogen

Bacterial pathogen *X. citri* pv. *citri* was isolated on Nutrient Agar medium from infected leaves and fruits collected from Horticulture farm, B. A. College of Agriculture and incubated at 28 °C. The pure culture of the bacteria was obtained by streak plate method. A single bacterial colony isolated on Nutrient agar medium which showed the characteristic features of *X. citri* pv. *citri* was picked up and streaked on sterilized plates containing pre-sterilized NA medium to obtain pure culture of isolated bacteria. The plates were kept in incubator for 48 hrs at 30 ± 2 °C. Then it was kept inside refrigerator at 4 °C for further use.

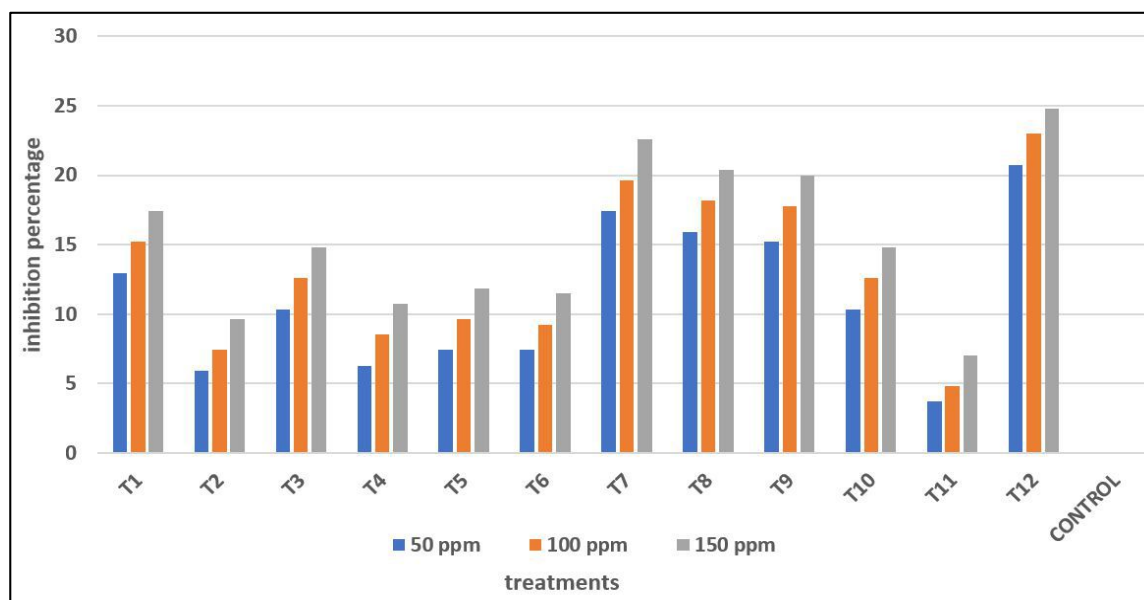
In vitro evaluation of allopathic antibiotics

All the antibiotics significantly reduced the growth of *X. citri* pv. *citri* as compared to control. The highest zone of inhibition was recorded in recommended check streptomycin sulphate 90% + tetracycline hydrochloride 10% SP with all three concentration (50, 100 and 150 ppm) 18.67, 20.67 and 22.33 mm respectively, the next best treatment in order of merit was T_7 i.e., Levofloxacin with all three concentrations with 15.67 mm, 17.67 mm, 20.33 mm inhibition zone followed by Ciprofloxacin with 14.33 mm, 16.33 mm, 18.33 mm (fig. 1) The lowest zone of inhibition was recorded in cefotaxime with all three concentrations i.e., 3.33 mm, 4.33 mm and 6.33 mm respectively followed by Gentamicin with 5.33 mm, 6.67 mm, and 8.67 mm respectively (Table 1).

Table 1: Evaluation of allopathic antibiotics against *X. citri* pv. *citri* *In vitro*

Tr. No.	Treatment ppm	Zone of inhibition (mm)			Inhibition over control (%)		
		50	100	150	50	100	150
1	Ampicillin 500 mg	11.67 ^d	13.67 ^d	15.67 ^d	12.97	15.18	17.41
2	Gentamicin 1 gm	5.33 ^g	6.67 ^g	8.67 ^g	5.92	7.41	9.63
3	Kanamycin 500 mg	9.33 ^e	11.33 ^e	13.33 ^e	10.36	12.5	14.81
4	Penicillin 600 mg	5.67 ^{fg}	7.67 ^{fg}	9.67 ^{fg}	6.30	8.50	10.74
5	Tetracycline 500 mg	6.67 ^f	8.67 ^f	10.67 ^f	7.41	9.60	11.86
6	Vancomycin 1 gm	6.67 ^f	8.33 ^f	10.33 ^f	7.41	9.25	11.48
7	Levofloxacin 500 mg	15.67 ^b	17.67 ^b	20.33 ^b	17.4	19.63	22.59
8	Ciprofloxacin 500 mg	14.33 ^c	16.33 ^c	18.33 ^c	15.9	18.14	20.37
9	Clarithromycin 500 mg	13.67 ^c	16.00 ^c	18.00 ^c	15.1	17.78	20
10	Azithromycin 500 mg	9.33 ^e	11.33 ^e	13.33 ^e	10.3	12.59	14.81
11	Cefotaxime 1 gm	3.33 ^h	4.33 ^h	6.33 ^h	3.7	4.81	7.03
12	Streptomycin sulphate (90%) + Tetracycline hypochloride (10%) (Recommended check)	18.67 ^a	20.67 ^a	22.33 ^a	20.7	22.90	24.81
13	Control	0.00 ⁱ	0.00 ⁱ	0.00 ⁱ	-	-	-
	S.E.m. $\pm$	0.32	0.35	0.35	-	-	-
	C.D. at 5%	0.93	1.01	1.01	-	-	-
	C.V. (%)	5.90	5.40	4.60	-	-	-

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

**Fig 1:** Efficacy of allopathic antibiotics against *X. citri* pv. *citri* *In vitro*

The present results of the study are in accordance with the findings of those reported earlier by several workers against *X. citri* pv. *citri*. the *In vitro* antibiotic sensitivity pattern of *X. axonopodis* was found 100 percent resistant to Cefotaxime (Islam *et al.*, 2014) [6]. The highest zone of inhibition was observed under streptocycline (25.73 mm) followed by chloramphenicol (23.10 mm) and oxytetracycline hydrochloride (22.80 mm) (Mondal *et al.*, 1980) [13]. The different chemicals against pathogen *X. axonopodis* pv. *citri* *In vitro* showed highest zone of inhibition under treatment Streptocycline (27.5 mm) (Negi & Kumar 2015) [14]. Nine antibiotics Levofloxacin, Streptomycin, Ampicillin, kanamycin, Erythromycin, Ciprofloxacin, Clarithromycin, Azithromycin, Amoxicillin

were tested against Xac by using inhibition zone technique under Completely Randomized Design (CRD). Out of these nine, Levofloxacin expressed best result by producing 14.22 mm mean inhibition zone followed by Ciprofloxacin (13.18 mm) (Hameed *et al.*, 2022) [4].

***In vitro* evaluation of Homeopathic medicines**

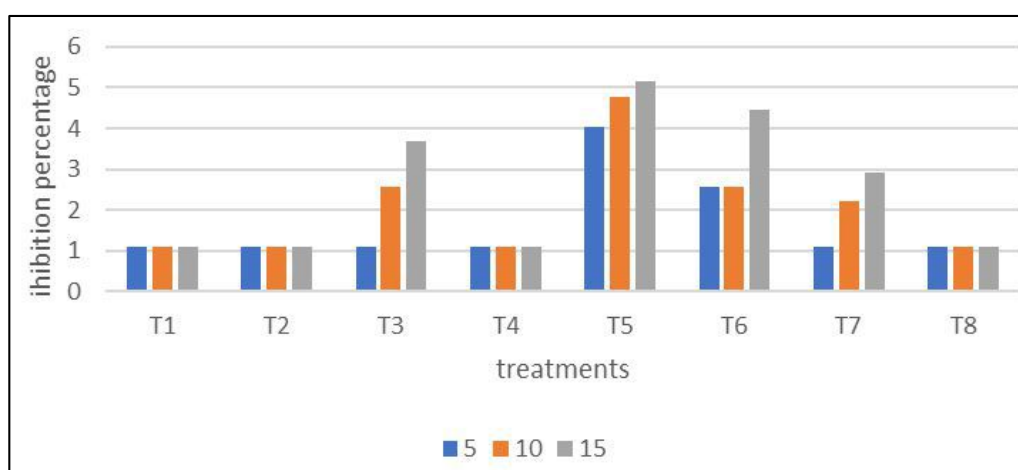
The highest zone of inhibitions were recorded in Lachesis 30 CH with three concentration, 5% (3.65 mm), 10% (4.28 mm) and 15% (4.62 mm) followed by phosphorus with 2.31 mm, 2.31 mm and 4.00 mm at 5, 10 and 15 percent concentration, respectively (fig. 2). Other six treatments were non-significant in reducing the growth of *X. citri* pv. *citri* (Table 2).

Table 2: Evaluation of homeopathic medicines against *X. citri* pv. *citri* In vitro

Tr. No.	Treatments %	Zone of inhibition (mm)			Inhibition over control (%)		
		5	10	15	5	10	15
1	Argentum nitricum 30 CH	1.00 ^c (1.00)	1.00 ^c (1.00)	1.00 ^d (1.00)	1.11	1.11	1.11
2	Calcium carbonicum 30 CH	1.00 ^c (1.00)	1.00 ^c (1.00)	1.00 ^d (1.00)	1.11	1.11	1.11
3	Carbo animalis 30 CH	1.00 ^c (1.00)	1.52 ^b (2.31)	1.82 ^b (3.31)	1.11	2.57	3.68
4	Equisetum 30 CH	1.00 ^c (1.00)	1.00 ^c (1.00)	1.00 ^d (1.00)	1.11	1.11	1.11
5	Lachesis 30 CH	1.91 ^a (3.65)	2.07 ^a (4.28)	2.16 ^a (4.62)	4.05	4.76	5.14
6	Phosphorus 30 CH	1.52 ^b (2.31)	1.52 ^b (2.31)	2.00 ^{ab} (4.00)	2.57	2.57	4.44
7	Zincum sulfuricum 30 CH	1.00 ^c (1.00)	1.41 ^b (1.99)	1.62 ^c (2.62)	1.11	2.21	2.92
8	Control	1.00 ^c (1.00)	1.00 ^c (1.00)	1.00 ^d (1.00)	1.11	1.11	1.11
S.E.m. ±		0.049	0.060	0.056	-	-	-
C.D. at 5%		0.147	0.179	0.169	-	-	-
C.V. (%)		7.20	7.87	6.73	-	-	-

Note: 1. Figures in parantheses are retransformed values and those outside are ($\sqrt{X + 1}$) transformed values

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

**Fig 2:** Efficacy of homeopathic medicines against *X. citri* pv. *citri* In vitro

The present results of the study are in accordance with screening of Argentum nitricum 30x, Calcium carbonicum 30x, Equisetum 30x against *Pseudomonas syringae* and observed there is no significant treatment effect on pathogen (Shah-Rossi *et al.*, 2009) [17]. Phosphorus at 9 CH and 3 CH showing growth inhibition of *Aspergillus niger* the phytopathogenic agent responsible for sisal red rot 14 and 21 percent, respectively (Gama *et al.*, 2015) [2].

These findings may be useful in controlling of citrus canker which has already become a severe threat to the citrus production. However, it is necessary to carry out field experiments to prove the effectiveness of these antibiotics because there are many factors in the field which influence the chemical control of plant diseases. Further studies for chemical disease control in field conditions are needed.

Conclusion

The use of allopathic antibiotics and homeopathic medicines might play an important role for the management of holistic bacterial citrus canker which is increasing day by day to avoid the severe epidemics of the disease which cannot be ruled out in future.

Further Research: Need to carry out field experiments to prove the effectiveness of these antibiotics

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

Statement of 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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