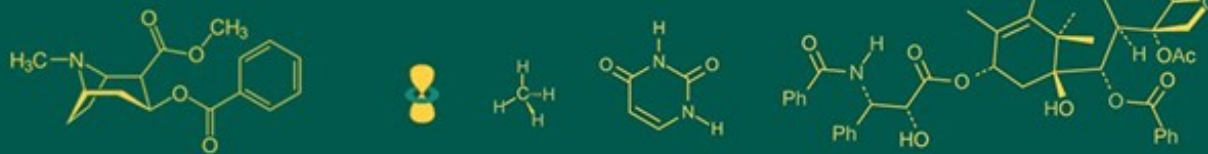


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In vitro evaluation of antibacterial activity of chemicals and essential oils against *Xanthomonas citri* pv. *citri* causing citrus canker

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ABSTRACT

Citrus canker, caused by *Xanthomonas citri* pv. *citri* (Xcc), is one of the most destructive bacterial diseases of citrus, causing considerable yield and quality losses worldwide. Prolonged reliance on copper-based bactericides and antibiotics such as streptomycin for its management has raised concerns over resistance development, phytotoxicity and environmental accumulation, creating a need to evaluate safer alternatives. The present study evaluated the *in vitro* antibacterial activity of six chemicals (Streptomycin sulphate, Ampicillin, Kasugamycin, Chloramphenicol, Tetracycline and Copper oxychloride, each at 250 and 500 ppm) and seven essential oils (Citronella, Clove, *Mentha*, Cardamom, Eucalyptus, Garlic and Ginger oil, each at 1000 and 2000 ppm) against Xcc by the paper disc diffusion method. Among the chemicals, Streptomycin sulphate recorded the maximum inhibition zone at both 250 ppm (25.53 mm) and 500 ppm (27.30 mm), significantly superior to all other treatments, followed by Tetracycline (19.13 mm and 23.13 mm), Chloramphenicol (18.20 mm and 22.40 mm) and Ampicillin (18.60 mm and 21.57 mm), while Copper oxychloride (13.50 mm and 19.00 mm) and Kasugamycin (13.50 mm and 19.53 mm) were the least effective. Among the essential oils, Garlic oil was most inhibitory at 1000 ppm (15.33 mm) and 2000 ppm (18.73 mm), at par with Clove oil (14.53 and 18.33 mm), while Ginger oil (11.47 mm and 14.27 mm) was the least effective. Inhibition zones of all treatments increased with concentration, confirming a dose-dependent antibacterial response. Streptomycin sulphate remains the most effective chemical, while Garlic and Clove oils show promise as eco-friendly components of an integrated citrus canker management programme.

Keywords: Citrus canker, *Xanthomonas citri* pv. *citri*, Chemicals, Essential oils, Inhibition zone, Antibacterial activity

1. Introduction

Citrus (*Citrus* spp.) is one of the most economically important fruit crops in the world, valued for its nutritional and commercial importance. In India, citrus ranks third among fruit crops after banana and mango, being cultivated on about 1.115 million hectares with an annual production of around 14.575 million tonnes and an average productivity of 13.07 t ha⁻¹ (Government of India, 2025) [1]. Maharashtra is one of the leading citrus-growing states of the country, with the crop occupying nearly 0.241 million hectares and yielding 2.156 million tonnes at a productivity of 8.95 t ha⁻¹ (Government of Maharashtra, 2025) [2].

Citrus cultivation, however, is severely constrained by biotic stresses, among which citrus canker caused by *Xanthomonas citri* pv. *citri* (Xcc) is one of the most destructive bacterial diseases. The disease is believed to have originated in South and South-East Asia, particularly India, before spreading to citrus-growing regions worldwide (Fawcett *et al.*, 1933) [3] (Civerolo, 1984) [4]. It was first recorded in Florida (USA) in 1915 and in India from Punjab in 1942, and has since been reported from almost every citrus-growing state in the country (Das, 2003) [5]. Xcc is a gram-negative, aerobic, rod-shaped bacterium with high host specificity; five distinct pathogenic groups (A, A*, Aw, B and C) have been described on the basis of host range and geographic distribution (Vauterin *et al.*, 1995) [6] (Verniere *et al.*, 1998) [7] (Ma *et al.*, 2021) [8]. The pathogen affects leaves, twigs and fruits, producing raised corky lesions that reduce fruit quality, cause premature fruit drop and lower market value, thereby inflicting substantial economic losses on growers (Timmer *et al.*, 2000) [9].

Since no commercially cultivated citrus cultivar possesses complete resistance to canker, its management has largely relied on cultural practices such as use of pathogen-free planting material, windbreaks and sanitation, supplemented by repeated foliar sprays of copper-based bactericides, sometimes combined with mancozeb or antibiotics such as streptomycin. However, prolonged and indiscriminate use of copper compounds has led to the emergence of copper-tolerant strains of Xcc, progressive accumulation of copper in orchard soils, phytotoxicity in sensitive cultivars and non-target toxicity to soil and aquatic organisms, while the agrical

tural use of antibiotics is being increasingly restricted worldwide owing to concerns over antimicrobial resistance. These limitations have created a pressing need for safer and more sustainable alternatives for managing this disease.

Plant-derived essential oils have attracted growing scientific interest as eco-friendly antibacterial agents in recent years. Being complex mixtures of volatile terpenes, terpenoids and phenylpropanoids, essential oils exhibit broad-spectrum antimicrobial activity by disrupting bacterial cell membranes and interfering with vital cellular processes, and are generally biodegradable and of low mammalian toxicity, making them attractive components of integrated disease management programmes. A comparative evaluation of conventional antibacterial chemicals alongside essential oils can therefore help identify effective and relatively safer options for management of citrus canker. Keeping this in view, the present investigation was undertaken to evaluate the *in vitro* antibacterial activity of selected chemicals and essential oils against Xcc, the causal agent of citrus canker.

2. Materials and Methods

2.1 Experimental site and period

The laboratory experiments were conducted during 2025-26 at the Department of Plant Pathology and Agricultural Microbiology, Govt. College of Agriculture, Pune, Maharashtra.

2.2 *Xanthomonas citri* pv. *citri* isolate

A pure and pathogenicity-confirmed isolate of *Xanthomonas citri* pv. *citri*, obtained from naturally infected citrus leaves collected from the Horticulture Farm of Govt. College of Agriculture, Pune and identified on the basis of morphological, cultural and biochemical characters, was used as the test pathogen throughout the study. The culture was maintained and multiplied on Nutrient Agar (NA) medium (Peptone 5 g, Beef extract 3 g, Agar 15 g, Distilled water 1000 ml) at $28 \pm 2^\circ\text{C}$.

2.3 Chemicals and essential oils evaluated

Six chemicals, viz., Streptomycin sulphate, Ampicillin (Amoxicillin trihydrate), Kasugamycin, Chloramphenicol, Tetracycline and Copper oxychloride, each tested at 250 and 500 ppm (Table 1) and seven essential oils, viz., Citronella, Clove, *Mentha*, Cardamom, Eucalyptus, Garlic and Ginger oil, each tested at 1000 and 2000 ppm (Table 2), were evaluated for antibacterial activity against the test pathogen.

2.4 In vitro evaluation of chemicals and essential oils: modified paper disc assay method

Sensitivity of the isolate to the test chemicals and essential oils was determined by the modified paper disc assay method (Bauer *et al.*, 1985) [10] (Hewitt *et al.*, 1989) [11] (Gakuubi *et al.*, 2016) [12]. The bacterium was multiplied in 100 ml nutrient broth in 250 ml conical flasks and incubated at $28 \pm 2^\circ\text{C}$ for 72 hours. A 20 ml aliquot of this bacterial suspension was mixed thoroughly with 1000 ml of molten, cooled ($28 \pm 2^\circ\text{C}$) nutrient agar, and the seeded medium was poured into sterilized Petri plates and allowed to solidify. Sterile filter paper discs (Whatman No. 42, 5 mm diameter) were soaked for 5 minutes in solutions of the test chemicals prepared at 250 and 500 ppm and essential oils at 1000 and 2000 ppm placed aseptically on the surface of the seeded medium. A disc soaked in sterile distilled water served as control. The plates were incubated at $28 \pm 2^\circ\text{C}$ for 72 hours and the diameter of the clear zone of inhibition around each disc was measured in millimetres. The activity index of each treatment was calculated as:

$$\text{Activity index} = \frac{\text{Inhibition zone of test sample}}{\text{Inhibition zone of the standard}}$$

2.5 Experimental design and statistical analysis

Both experiments were laid out in a Completely Randomized Design (CRD) with three replications per treatment. Data on the diameter of the inhibition zone were analyzed statistically to test the significance of treatment effects and standard error of mean [$SE_m \pm$] and critical

difference [CD @ 1%] were worked out for comparison of treatment means.

3. Results and Discussion

3.1 Antibacterial activity of chemicals against *Xanthomonas citri* pv. *citri* at 250 ppm

The data presented in Table 3 revealed that among the six chemicals tested at 250 ppm, Streptomycin sulphate recorded the maximum inhibition zone (25.53 mm) and activity index of 1.00 and was significantly superior to all other treatments, followed by Tetracycline (19.13 mm, 0.75), Ampicillin (18.60 mm, 0.73) and Chloramphenicol (18.20 mm, 0.71). Kasugamycin and Copper oxychloride recorded the lowest and statistically similar inhibition zones (13.50 mm each, activity index 0.53), while the untreated control showed no inhibition. Kale *et al.* (1994) [13] similarly reported Streptomycin sulphate to be more effective than Copper oxychloride in the chemical management of citrus canker and Bhure *et al.* (2019) [14] recorded higher inhibition with antibiotic treatments than with a copper-based treatment against *X. axonopodis* pv. *citri*. The comparatively lower activity of Copper oxychloride may be attributed to its mode of action as a broad-spectrum, multi-site protoplasmic poison that depends on slow release of cupric ions and has limited diffusibility through agar, unlike readily diffusible antibiotic molecules such as streptomycin and tetracycline that act rapidly at the bacterial ribosome.

3.2 Antibacterial activity of chemicals against *Xanthomonas citri* pv. *citri* at 500 ppm

At 500 ppm (Table 4), Streptomycin sulphate again recorded the highest inhibition zone (27.30 mm, activity index 1.00), followed by Tetracycline (23.13 mm, 0.85), Chloramphenicol (22.40 mm, 0.82) and Ampicillin (21.57 mm, 0.79), whereas Kasugamycin (19.53 mm, 0.71) and Copper oxychloride (19.00 mm, 0.69) were the least effective. A comparison of Tables 3 and 4 showed that the inhibition zone of every chemical increased with concentration, confirming a clear dose-dependent antibacterial response, consistent with the findings of Jadeja *et al.* (2000) [15] and Thind and Singh (2015) [16] against *X. axonopodis* pv. *citri*. Notably, the magnitude of increase between 250 and 500 ppm was comparatively smaller for Copper oxychloride (13.50 to 19.00 mm) than for the antibiotics, again reflecting its slower, diffusion-limited mode of action.

3.3 Antibacterial activity of essential oils against *Xanthomonas citri* pv. *citri* at 1000 ppm

Data presented in Table 5 showed that among the seven essential oils tested at 1000 ppm, Garlic oil recorded the highest inhibition zone (15.33 mm, activity index 1.00), which was at par with Clove oil (14.53 mm, 0.94), Citronella oil (13.43 mm, 0.87) and *Mentha* oil (13.27 mm, 0.86), followed by Cardamom oil (12.60 mm, 0.82) and Eucalyptus oil (11.80 mm, 0.77). Ginger oil recorded the lowest inhibition zone (11.47 mm, 0.75), while the control showed no inhibition. The strong antibacterial activity of Garlic oil may be attributed to its rich content of organosulfur compounds, principally allicin and diallyl disulfide, which react with thiol groups of bacterial enzymes and disrupt membrane integrity; comparable inhibitory activity of sulfur-rich essential oils against *Xanthomonas* spp. was reported by Bajpai *et al.* (2010) [17]. The moderate activity of Citronella and *Mentha* oils is consistent with the antibacterial potential of their major monoterpene constituents, as reported by Wonni *et al.* (2016) [18] against *Xanthomonas oryzae* pathogens.

3.4 Antibacterial activity of essential oils against *Xanthomonas citri* pv. *citri* at 2000 ppm

At 2000 ppm (Table 6), Garlic oil again recorded the highest inhibition zone (18.73 mm, activity index 1.00), followed by Clove oil (18.33 mm, 0.98), *Mentha* oil (17.20 mm, 0.92) and Citronella oil (16.27 mm, 0.87), all of which were statistically at par with Garlic oil, while Cardamom oil (15.30 mm, 0.81), Eucalyptus oil (14.57 mm, 0.78) and Ginger oil (14.27 mm, 0.76) were comparatively less effective.

tive. The consistent rise in the inhibition zone of all seven oils with increasing concentration confirmed a clear dose-dependent antibacterial response, a trend also reported by *Gakuubi et al. (2016)* [12] for *Tagetes minuta* essential oil against phytopathogenic bacteria. Taken together, the inhibition produced by Garlic and Clove oils at

2000 ppm approached the level achieved by several moderately active synthetic chemicals such as Ampicillin, Chloramphenicol and Tetracycline evaluated in the present study, indicating that these two oils merit further consideration as botanical components of an integrated management programme for citrus canker.

Table 1. Antibacterial activity of different chemicals against *Xanthomonas citri* pv. *citri* at 250 and 500 ppm

Treatment No.	Treatment details	250 ppm		500 ppm	
		Inhibition zone (mm)	Activity index	Inhibition zone (mm)	Activity index
T1	Streptomycin sulphate	25.53	1.00	27.30	1.00
T2	Ampicillin (Amoxicillin trihydrate)	18.60	0.73	21.57	0.79
T3	Kasugamycin	13.50	0.53	19.53	0.71
T4	Chloramphenicol	18.20	0.71	22.40	0.82
T5	Tetracycline	19.13	0.75	23.13	0.85
T6	Copper oxychloride	13.50	0.53	19.00	0.69
T7	Control	0.00	-	0.00	-

Table 2. Antibacterial activity of different essential oils against *Xanthomonas citri* pv. *citri* at 1000 and 2000 ppm

Treatment No.	Treatment details	1000 ppm		2000 ppm	
		Inhibition zone (mm)	Activity index	Inhibition zone (mm)	Activity index
T1	Citronella oil	13.43	0.87	16.27	0.87
T2	Clove oil	14.53	0.94	18.33	0.98
T3	Mentha oil	13.27	0.86	17.20	0.92
T4	Cardamom oil	12.60	0.82	15.30	0.81
T5	Eucalyptus oil	11.80	0.77	14.57	0.78
T6	Garlic oil	15.33	1.00	18.73	1.00
T7	Ginger oil	11.47	0.75	14.27	0.76
T8	Control	0.00	-	0.00	-

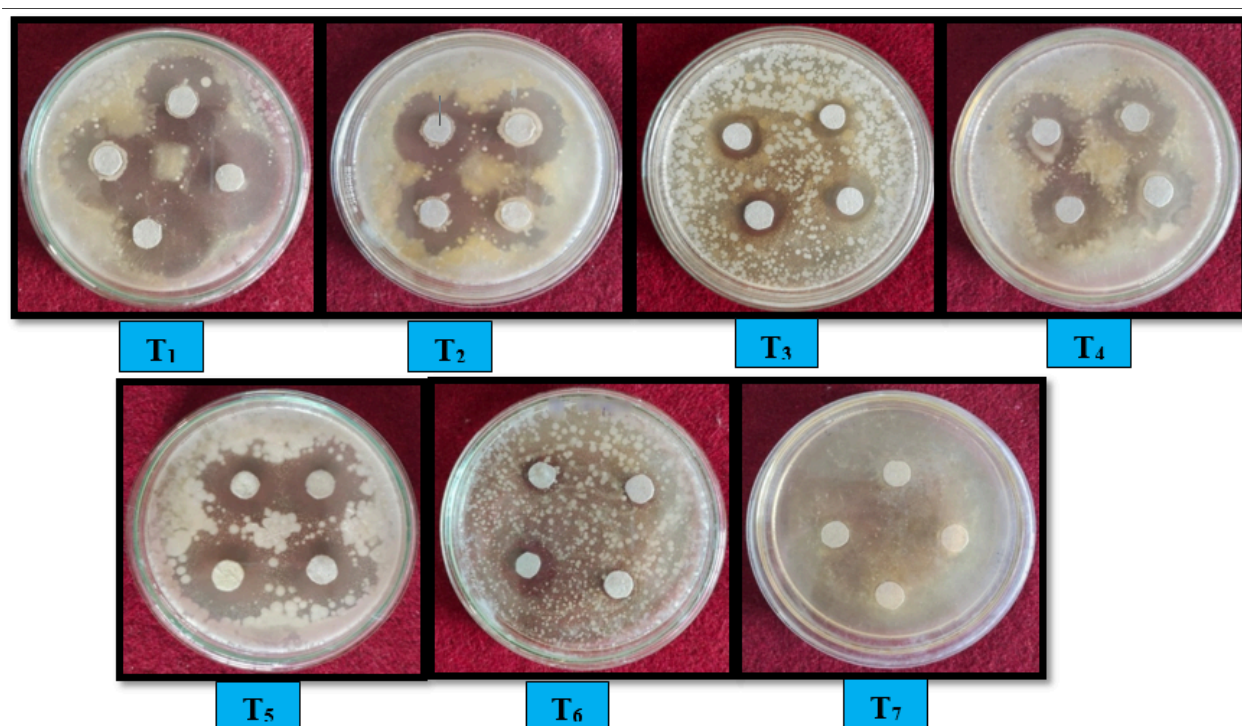


Plate 3.1. Effect of different chemicals against *Xanthomonas citri* pv. *citri* at 250 ppm

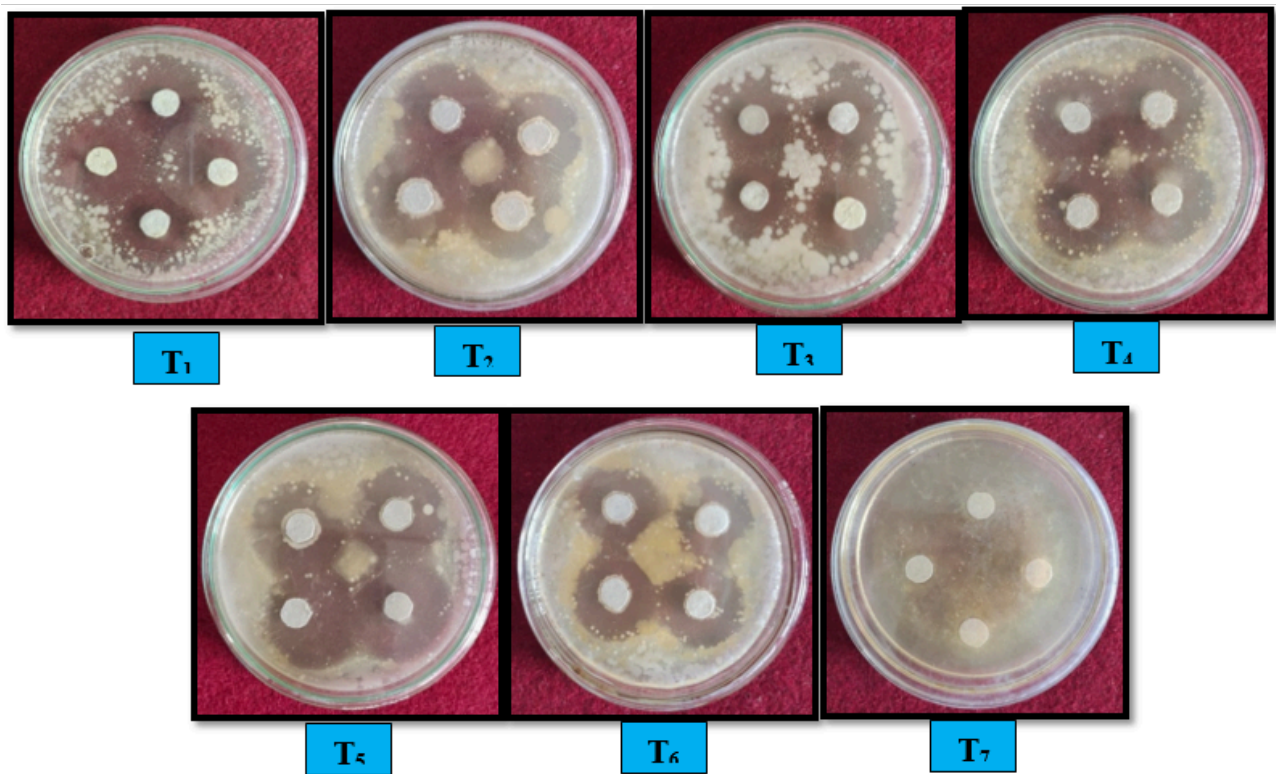


Plate 3.2. Effect of different chemicals against *Xanthomonas citri* pv. *citri* at 500 ppm

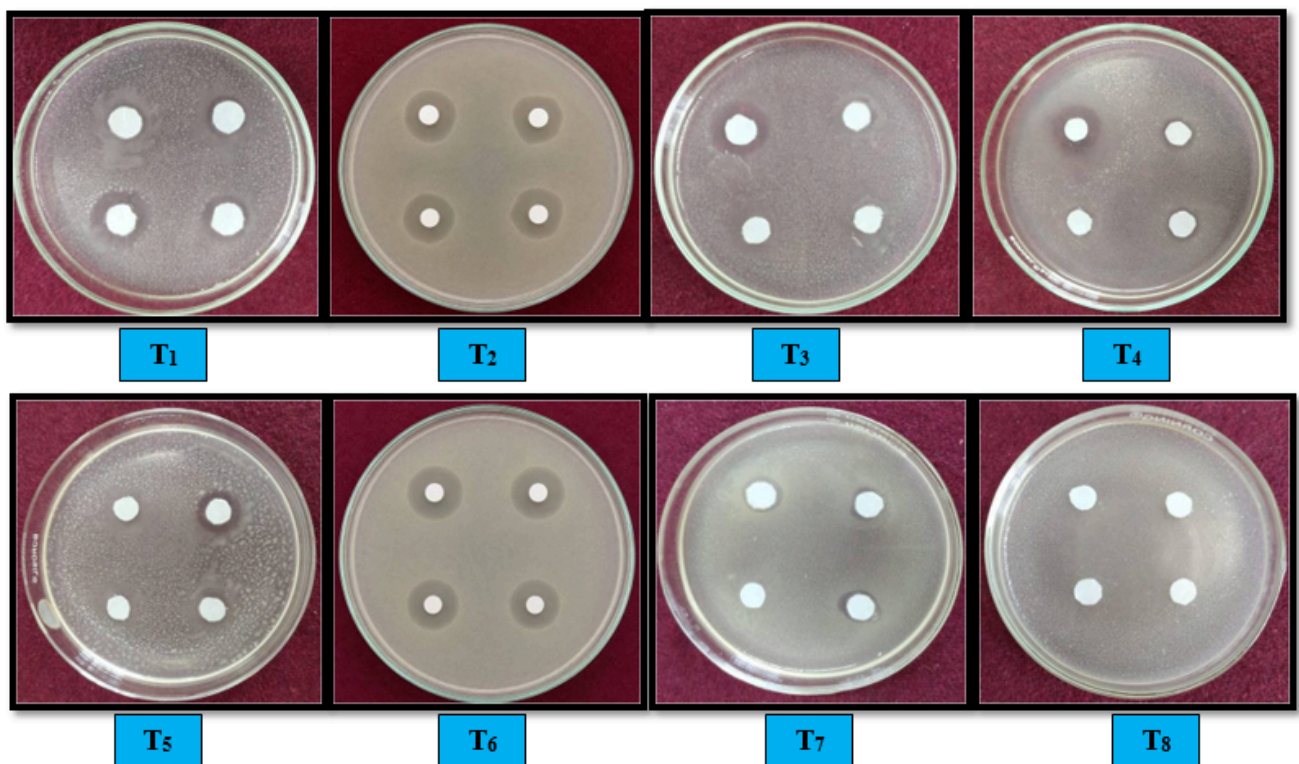


Plate 3.3. Effect of different essential oils against *Xanthomonas citri* pv. *citri* at 1000 ppm

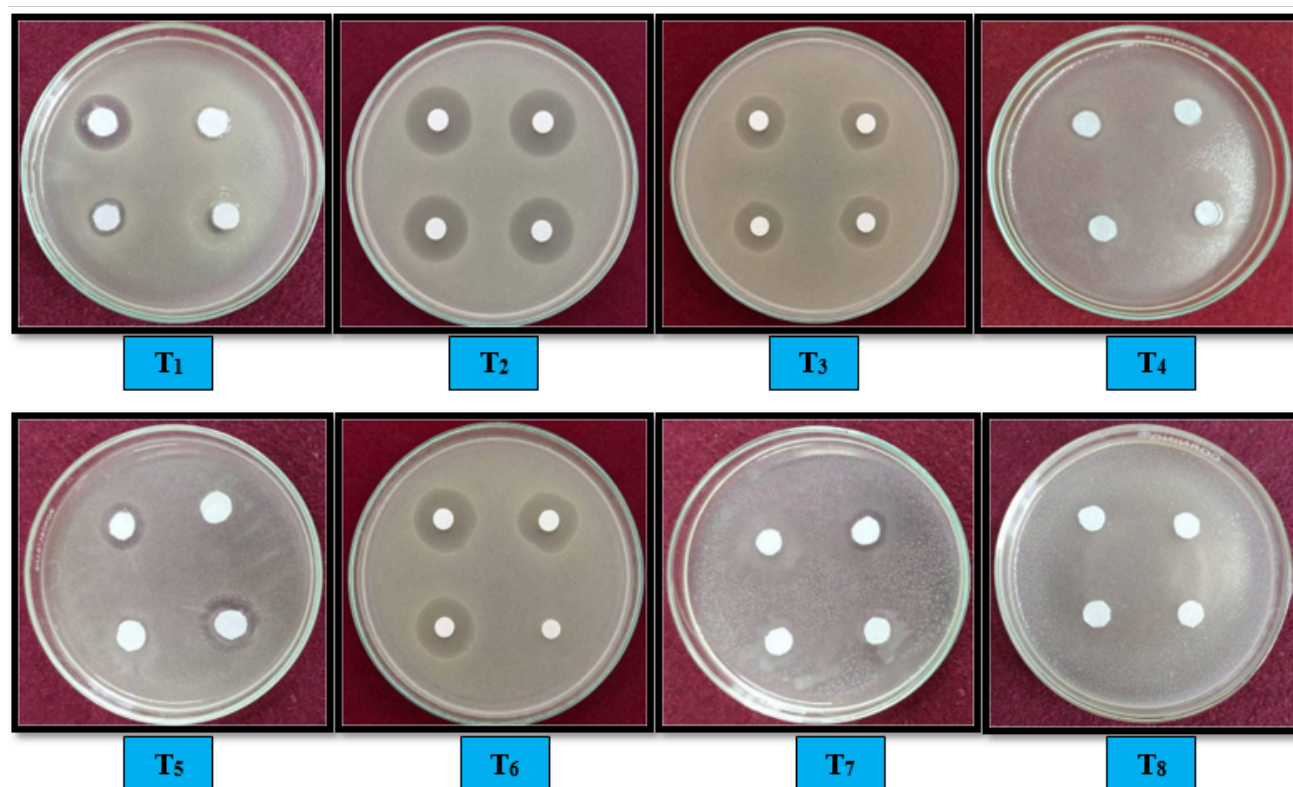


Plate 3.4. Effect of different essential oils against *Xanthomonas citri* pv. *citri* at 2000 ppm

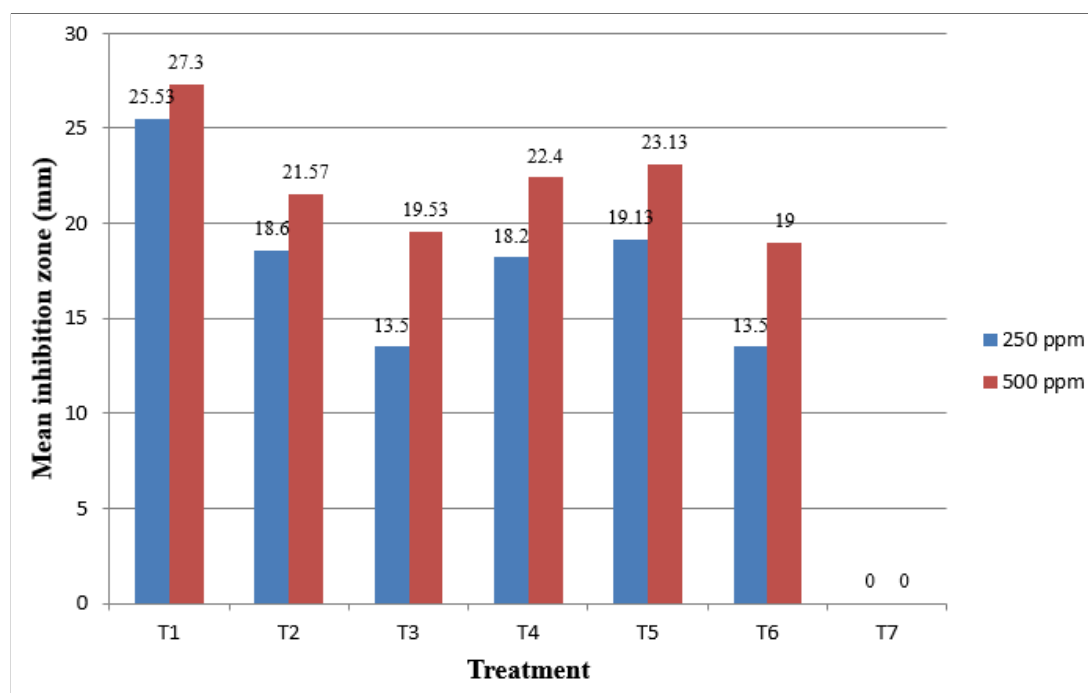


Figure 3.1. Effect of different chemicals against *Xanthomonas citri* pv. *citri* at 250 ppm

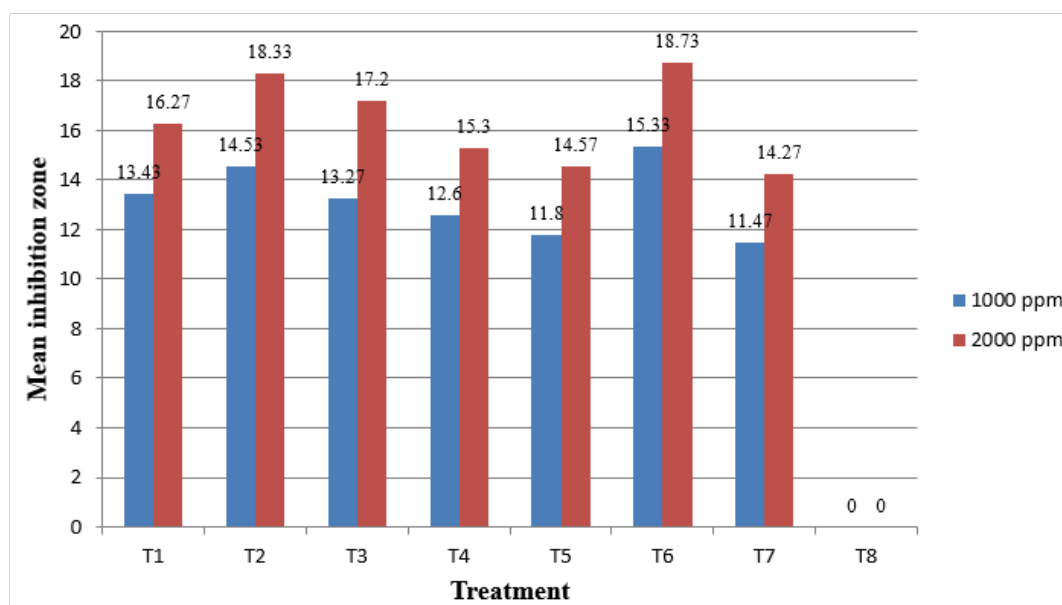


Figure 3.2. Effect of different chemicals against *Xanthomonas citri* pv. *citri* at 500 ppm

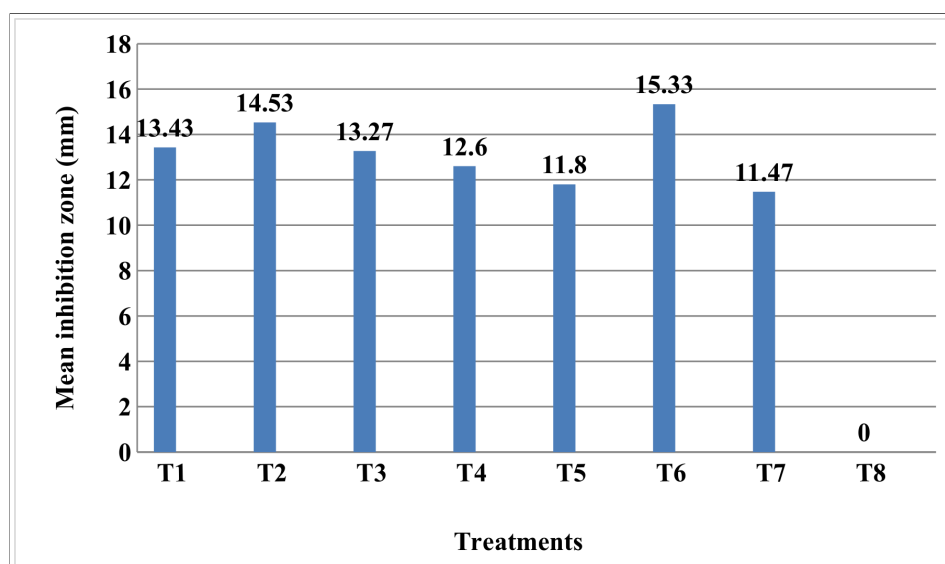


Figure 3.3. Effect of different essential oils against *Xanthomonas citri* pv. *citri* at 1000 ppm

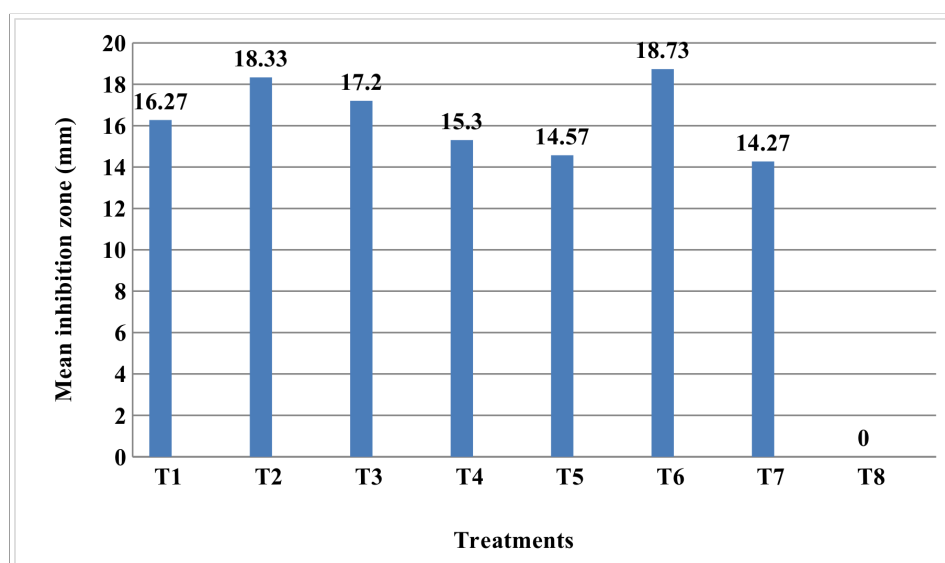


Figure 3.4. Effect of different essential oils against *Xanthomonas citri* pv. *citri* at 2000 ppm

4. Conclusion

Among the six chemicals evaluated against *Xanthomonas citri* pv. *citri*, Streptomycin sulphate proved to be the most effective at both 250 ppm (25.53 mm) and 500 ppm (27.30 mm), followed by Tetracycline, Chloramphenicol and Ampicillin, while Copper oxychloride and Kasugamycin were the least effective. Among the seven essential oils tested, Garlic oil recorded the highest antibacterial activity at both 1000 ppm (15.33 mm) and 2000 ppm (18.73 mm) and was at par with Clove oil, whereas Ginger oil was the least effective. Inhibition zones of all treatments, both chemicals and essential oils, increased with an increase in concentration, confirming a clear dose-dependent antibacterial response. The results indicate that while Streptomycin sulphate remains the most effective chemical option, Garlic and Clove oils show promising antibacterial potential broadly comparable to the moderately active synthetic chemicals, suggesting their scope as eco-friendly components of an integrated citrus canker management programme.

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