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In Vitro Evaluation Of Antimicrobial Activity Of Selected Plant Extracts And Antibiotics Against *Xanthomonas Axonopodis* Pv. *Citri*, The Causal Agent Of Citrus Canker

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Abstract

The antimicrobial activity of selected plant extracts, including *Azadirachta indica, Ocimum sanctum, Calotropis procera, Chrysanthemum cinerariifolium, Ipomoea carnea, Datura stramonium, Zingiber officinale, Curcuma longa, Catharanthus roseus, Nicotiana tabacum, Annona squamosa, Aspergillus racemosus, Argemone mexicana,* and *Jatropha* species, was evaluated *in vitro* against *Xanthomonas axonopodis* pv. *citri*, the causal agent of citrus canker disease. Among the tested plant extracts, *Argemone mexicana* exhibited the highest growth inhibition (2.6 cm), while *Ipomoea carnea* showed the lowest (0.9 cm). Additionally, the antimicrobial activity of selected antibiotics, including Tetracycline, Ampicillin, Amoxicillin, Levofloxacin, Ofloxacin, Terramycin, Cefixime, Penicillin, Azithromycin, and Cefadroxil, was tested *in vitro* against the same pathogen. Levofloxacin demonstrated the highest growth inhibition (3.0 cm), whereas Ampicillin and Penicillin exhibited the lowest inhibition (1.5 cm). These findings highlight the potential of plant extracts and antibiotics in managing citrus canker disease.

Keywords: Plant extracts, Antibiotics, Citrus canker, *Xanthomonas axonopodis pv. citri.*, citrus canker, natural antimicrobials, medicinal plants, disease management.

INTRODUCTION

Citrus is one of the most widely cultivated fruit crops globally, grown in over 130 countries, primarily in tropical and subtropical regions (Gill et al., 2022). With an estimated global production of 150 million tonnes across more than 14.4 million hectares (FAO, 2022), citrus plays a vital role in the global economy. India ranks as the fifth-largest citrus producer, with an annual production of approximately 13,976 thousand metric tonnes cultivated over 1,054 thousand hectares (Gill et al., 2022, 2023). Major citrus-producing states in India include

Maharashtra, Punjab, Tamil Nadu, Andhra Pradesh, Karnataka, Madhya Pradesh, Assam, Uttar Pradesh, and Rajasthan (Das, 2003). However, citrus production faces significant challenges due to low yields, high marketing costs, and susceptibility to various diseases (Gottwald et al., 2002).

Among citrus diseases, citrus canker, also known as citrus bacterial spot or citrus bacteriosis, is one of the most devastating. It is caused by the bacterium *Xanthomonas axonopodis* pv. *citri* and affects nearly all economically important citrus varieties (Rossetti, 1977; Dopson, 1964). The disease manifests as raised, corky lesions on leaves, stems, and fruit, reducing both yield and fruit quality. Symptoms typically appear within 14 days of infection, and the disease spreads rapidly through wind-driven rain, contaminated tools, and infected nursery stock (Patel & Desai, 1970; Nirvan, 1961).

Managing citrus canker is challenging, as traditional control measures, including the use of copper-based bactericides and antibiotics, have shown limited long-term effectiveness due to bacterial resistance and environmental concerns. Disease prevention strategies primarily rely on the use of disease-free nursery stock, eradication of infected trees, and the development of canker-resistant citrus varieties (Ismail and Zhang 2004; Graham et al 2004; Ali et al 2023; Gautam et al 2024). Given these challenges, alternative approaches such as plant-derived antimicrobial compounds are being explored for sustainable disease management (Davies and Davies.2010; Baym et al 2015; Pandey and Kumar 2013; S. Vaou et al 2021). This study aims to evaluate the in vitro antimicrobial activity of selected plant extracts and antibiotics against *X. axonopodis* pv. *citri*. The findings will help identify potential bio-based alternatives for controlling citrus canker, contributing to environmentally friendly and effective disease management strategies.

MATERIAL AND METHODS

The methodology employed in the study of citrus canker disease management involved several systematic steps to isolate and test the pathogen *Xanthomonas axonopodis pv. citri*, as well as to evaluate the efficacy of various plant extracts and antibiotics. Here's a thorough breakdown of the material and methods used:

- **Collection of Samples**: Infected citrus fruits exhibiting red or black spots were collected from fields located in the Wardha and Amravati districts of Maharashtra. This ensured that the samples were representative of the local infection conditions and provided a reliable source for isolating the pathogen.
- **Isolation of Pathogen**: The infected parts of the collected fruits were subjected to a process of serial dilution. The diluted samples were then streaked onto standardized media, which consisted of peptone (5.0 g), beef extract (3.0 g), dextrose (10.0 g), distilled water (1000 ml), and agar (15 g) with a pH of 7.2. This media composition was specifically designed to support the growth of *Xanthomonas axonopodis pv. citri*, facilitating its isolation from the infected fruit.
- **Preparation of Plant Extracts**: Various plant extracts were prepared for testing against the pathogen. Notably, *Argemone mexicana* was highlighted for its significant inhibitory effects. The extracts were diluted in distilled water to create different concentrations for testing.
- **Antibiotic Testing**: A range of antibiotics, including Levofloxacin, Ofloxacin, and Teramycine, were tested *in vitro* for their effectiveness against the growth of *Xanthomonas axonopodis pv. citri*. The antibiotics were applied in varying concentrations to determine their inhibitory action on the pathogen.
- **Evaluation of Inhibition**: The effectiveness of both plant extracts and antibiotics was measured by assessing the zone of inhibition around the application site on the media. For instance, *Argemone mexicana* showed a maximum zone of inhibition of 2.6 cm, while Levofloxacin exhibited a maximum inhibition of 3.0 cm

OBSERVATION AND RESULTS

Antimicrobial Activity of Plant Extracts

The antimicrobial activity of 14 plant extracts against *Xanthomonas axonopodis* pv. *citri*, the causative agent of citrus canker, was evaluated by measuring the zone of inhibition at three different concentrations (0.1 ml/ml, 0.2 ml/ml, and 0.5 ml/ml) in distilled water.

Among the tested plant extracts, *Argemone mexicana* exhibited the highest antibacterial activity, with inhibition zones increasing from 1.8 cm at 0.1 ml/ml to 2.3 cm at 0.5 ml/ml, indicating a strong dose-dependent effect. *Calotropis procera* displayed moderate inhibition, with zones ranging from 0.9 cm at the lowest concentration to 1.4 cm at the highest. *Datura stramonium* also showed antibacterial activity, though slightly weaker, with inhibition zones increasing from 0.8 cm to 1.1 cm as the concentration increased. *Ocimum sanctum* exhibited limited effectiveness, showing an inhibition zone of 0.8 cm only at the highest concentration (0.5 ml/ml).

In contrast, several plant extracts, including *Azadirachta indica* (Neem), *Chrysanthemum cinerariaefolium*, *Zingiber officinale* (Ginger), and *Curcuma longa* (Turmeric), failed to inhibit bacterial growth at any tested concentration. These findings suggest that while certain plant extracts exhibit antibacterial activity against *Xanthomonas axonopodis* pv. *citri*, others commonly associated with antimicrobial properties may not be effective under these experimental conditions.
The results also indicate a general trend of increased antibacterial activity with higher extract concentrations, as observed in *Argemone mexicana* and *Calotropis procera*. However, the lack of activity in several extracts underscores the pathogen's resistance to specific plant-derived bioactive compounds.

Antibacterial Activity of Antibiotics
The antibacterial efficacy of various antibiotics against *Xanthomonas axonopodis* pv. *citri* was assessed at five different concentrations (0.05 mg/ml, 0.5 mg/ml, 1.0 mg/ml, 1.5 mg/ml, and 2.0 mg/ml), with inhibition zones measured to determine effectiveness.
Levofloxacin demonstrated the highest efficacy, with inhibition zones increasing from 1.6 cm at 0.05 mg/ml to 3.0 cm at 2.0 mg/ml, indicating strong and consistent antibacterial activity. Ofloxacin and Teramycine followed closely, displaying inhibition zones ranging from 1.4 cm at 0.05 mg/ml to 2.7 cm at 2.0 mg/ml, making them viable alternatives to Levofloxacin.
Tetracycline exhibited moderate activity, with inhibition zones increasing from 1.0 cm at the lowest concentration to 2.6 cm at the highest. Cefixime showed a slower increase in efficacy, with inhibition zones ranging from 0.9 cm to 2.2 cm across the tested concentrations, suggesting a relatively weaker but still effective antibacterial response.
Amoxicillin and Azithromycin demonstrated limited effectiveness. Amoxicillin's inhibition zones ranged from 0.8 cm to 1.6 cm, while Azithromycin displayed minimal activity at lower concentrations, reaching a maximum inhibition zone of 2.0 cm at the highest concentration. Penicillin exhibited even weaker antibacterial activity, with inhibition zones ranging from 0.7 cm to 1.5 cm, indicating limited potential for managing citrus canker.
Notably, Ampicillin and Cefadroxil showed no inhibition at any tested concentration, indicating complete ineffectiveness against *Xanthomonas axonopodis* pv. *citri*. This suggests that the pathogen possesses inherent resistance to these antibiotics, making them unsuitable for disease control.
Overall, the study highlights Levofloxacin, Ofloxacin, and Teramycine as the most effective antibiotics for suppressing *Xanthomonas axonopodis* pv. *citri*, exhibiting strong dose-dependent antibacterial activity. Tetracycline and Cefixime demonstrated moderate effectiveness and may serve as supplementary treatment options. Conversely, the inefficacy of Ampicillin and Cefadroxil emphasizes the necessity of targeted antibiotic selection for effective disease management.

DISCUSSIONS

Studies have identified several plant extracts with antibacterial activity against *X. axonopodis* pv. *citri*. For instance, *Citrullus colocynthis* (C. colocynthis) and *Nigella sativa* (N. sativa) exhibited significant antibacterial activity, with maximum inhibition zones of 3.22 mm and 2.82 mm, respectively (Aslam et al., 2024).Phyto-extracts of garlic and moringa also showed promising results in controlling citrus canker disease caused by *X. axonopodis* pv. *citri* (Usama et al., 2023).A broad-spectrum antagonist strain, ZJLMBA1908, was identified with potent antibacterial activity against *X. citri*. This strain presents a promising new candidate for biological control of citrus canker, contributing to the potential biocontrol mechanisms of *Bacillus amyloliquefaciens* (Ke et al., 2023).In vitro studies have also tested the antibacterial properties of solvents such as petroleum ether, chloroform, ethyl acetate, dichloromethane, distilled water, and methanol. These solvents were used to extract compounds from plants like *Azadirachta indica* and *Eucalyptus globulus*, which showed antibacterial activity against *X. axonopodis* pv. *citri* (Gurav et al., 2022). Overall, managing citrus canker with antibiotics and chemicals has been shown to be effective in controlling disease incidence and improving fruit yield (Shahbaz et al., 2023).
Citrus canker, caused by the bacterium *Xanthomonas axonopodis* pv. *citri*, is a major threat to citrus cultivation, leading to severe economic losses due to lesions on leaves, stems, and fruits, ultimately causing premature fruit drop (Gottwald et al., 2002). Effective management strategies are essential to mitigate its impact, and previous research has demonstrated the potential of plant-based antimicrobial agents. Studies by Manonmani et al. (2009) have reported the bactericidal activity of various plant extracts, including *Acalypha indica*, *Achyranthes aspera*, *Aloe vera*, *Azadirachta indica*, *Datura metel*, *Hibiscus rosa-sinensis*, *Nerium oleander*, *Ocimum sanctum*, *Ocimum basilicum*, *Phyllanthus emblica*, *Polyalthia longifolia*, *Piper betle*,

Punica granatum, *Solanum torvum*, and *Solanum trilobatum*. Among these, *Polyalthia longifolia* exhibited notable inhibitory activity against *Xanthomonas axonopodis* pv. *citri*.
The antimicrobial properties of plant extracts have been widely documented, highlighting their antibacterial, antiviral, antifungal, anthelmintic, antimolluscal, and anti-inflammatory effects (Palombo et al., 2006). In this study, *Xanthomonas axonopodis* pv. *citri* was first isolated from infected citrus fruits, characterized, and subsequently subjected to in vitro antimicrobial testing using selected plant extracts and antibiotics.
Among the tested plant extracts, *Argemone mexicana* demonstrated the highest antibacterial activity, with inhibition zones increasing from 1.8 cm at 0.1 ml/ml to 2.3 cm at 0.5 ml/ml, indicating a strong dose-dependent effect. *Calotropis procera* displayed moderate inhibition, with zones ranging from 0.9 cm at the lowest concentration to 1.4 cm at the highest. *Datura stramonium* also showed antibacterial activity, with inhibition zones increasing from 0.8 cm to 1.1 cm as the concentration increased. *Ocimum sanctum* exhibited limited effectiveness, showing an inhibition zone of 0.8 cm only at the highest concentration (0.5 ml/ml).

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Notably, Ampicillin and Cefadroxil showed no inhibition at any tested concentration, indicating complete ineffectiveness against *Xanthomonas axonopodis* pv. *citri*. This suggests that the pathogen possesses inherent resistance to these antibiotics, making them unsuitable for disease control.

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CONCLUSION

This study provides strong evidence of the antimicrobial efficacy of selected plant extracts and antibiotics against *Xanthomonas axonopodis* pv. citri, the causative agent of citrus canker. Among the tested plant extracts, Argemone mexicana exhibited the highest antibacterial activity, achieving a maximum inhibition zone of 2.6 cm at a concentration of 2 ml/ml, demonstrating significant bactericidal potential. *Calotropis procera* and *Datura stramonium* showed moderate inhibitory effects, while other extracts, including *Azadirachta indica*, displayed no measurable activity under the experimental conditions.

The antibiotic assay identified Levofloxacin as the most potent antimicrobial agent, with a maximum inhibition zone of 3.0 cm at 2 mg/ml, followed by Ofloxacin and Teramycine, both of which exhibited strong dose-dependent antibacterial effects. In contrast, Ampicillin and Cefadroxil showed no inhibition, suggesting an inherent resistance of *Xanthomonas axonopodis* pv. citri to these antibiotics.

These findings underscore the potential of *Argemone mexicana* as a promising biopesticide and Levofloxacin as a highly effective antibiotic for managing citrus canker. Their demonstrated efficacy supports their incorporation into integrated disease management strategies, reducing dependency on synthetic chemicals and promoting sustainable agricultural practices. Future research should focus on validating these results through field trials, investigating possible synergistic interactions between plant-based and antibiotic treatments, and developing formulations optimized for large-scale agricultural applications to enhance citrus canker management.

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Fig.1 Isolated pathogen

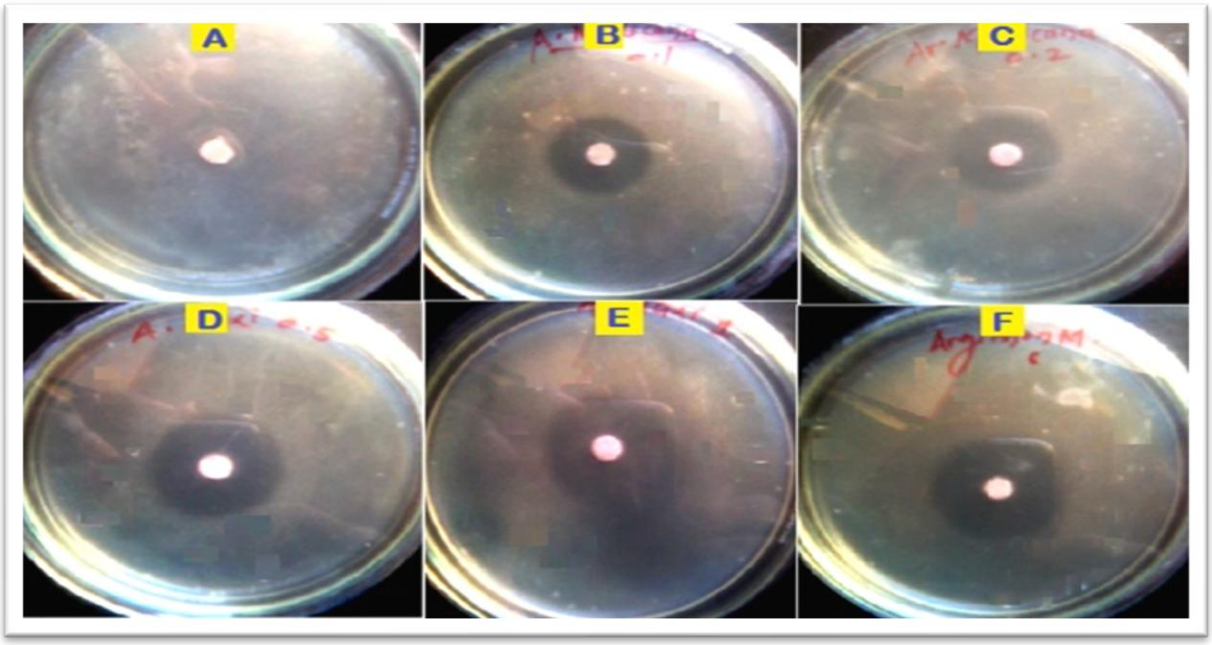

Fig. 2 *Argemone mexicana* plant extract showing zone of inhibition against *Xanthomonas axonopodis*

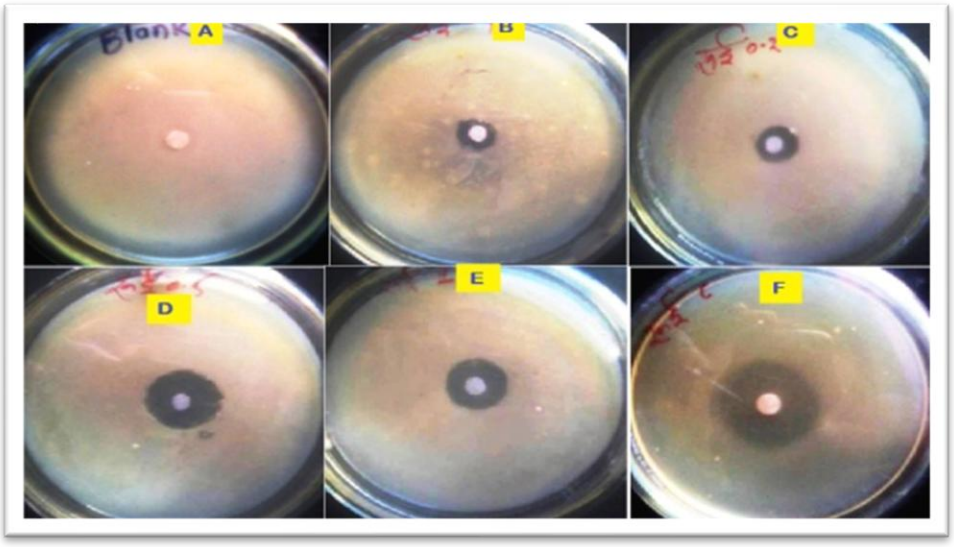

Fig. 3 *Calotropis procera* plant extract showing zone of inhibition against *Xanthomonas axonopodis*

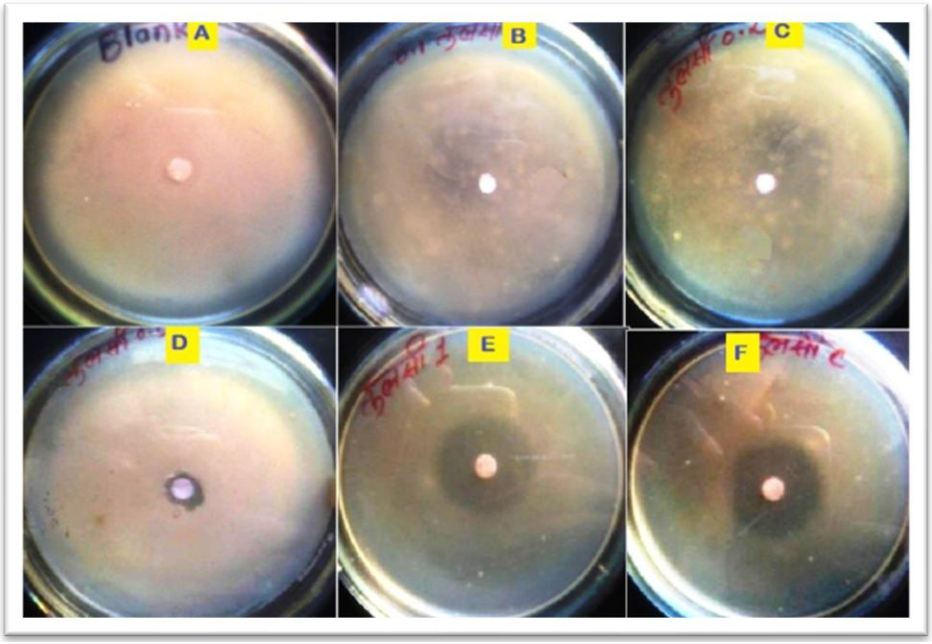

Fig. 4 *Ocimum sanctum* plant extract showing zone of inhibition against *Xanthomonas axonopodis*

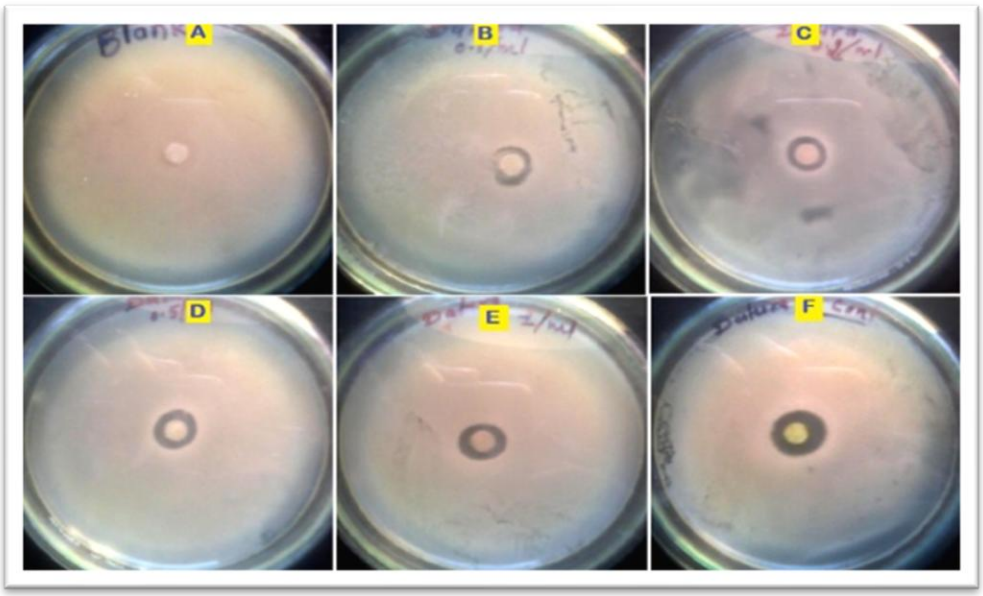

Fig.5 *Dhatura stramonium* plant extract showing zone of inhibition against *Xanthomonas axonopodis*

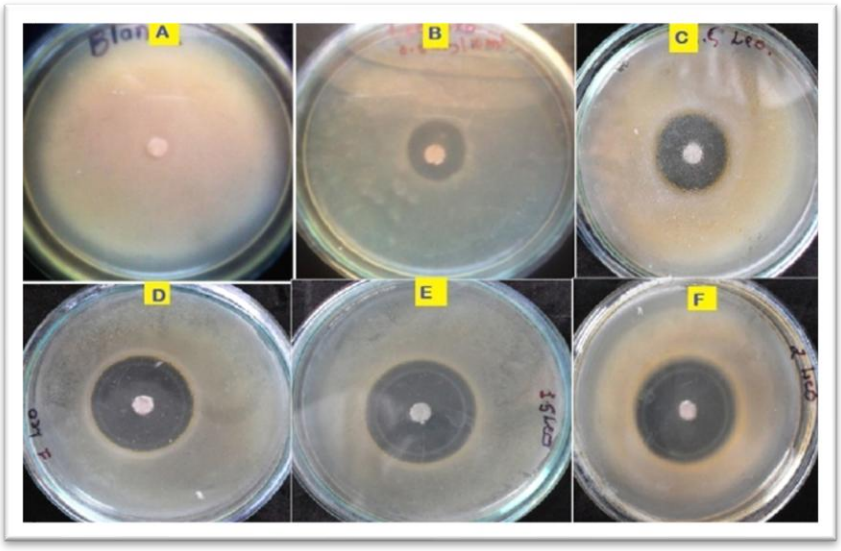

Fig 6 Levofloxacin show zone of inhibition against *Xanthomonas axonopodis*.

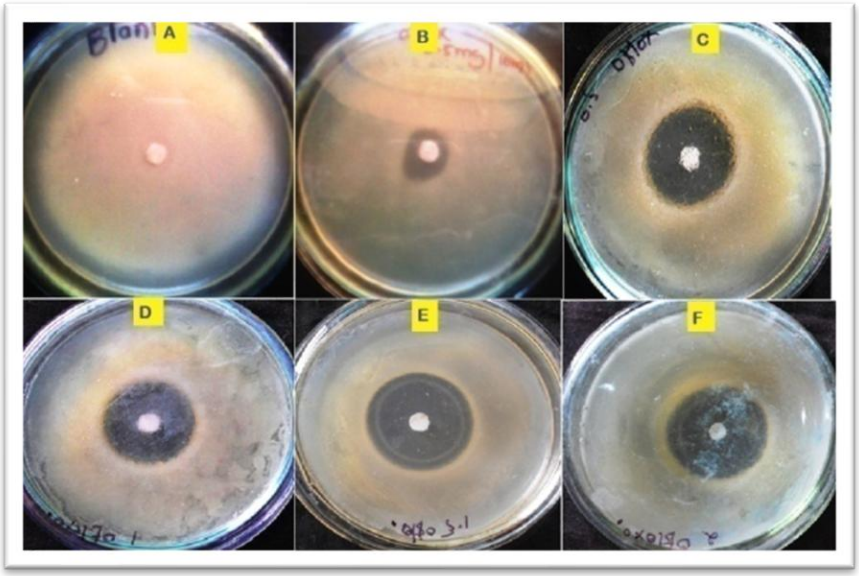

Fig 7 Ofloxacin show zone of inhibition against *Xanthomonas axonopodis*.

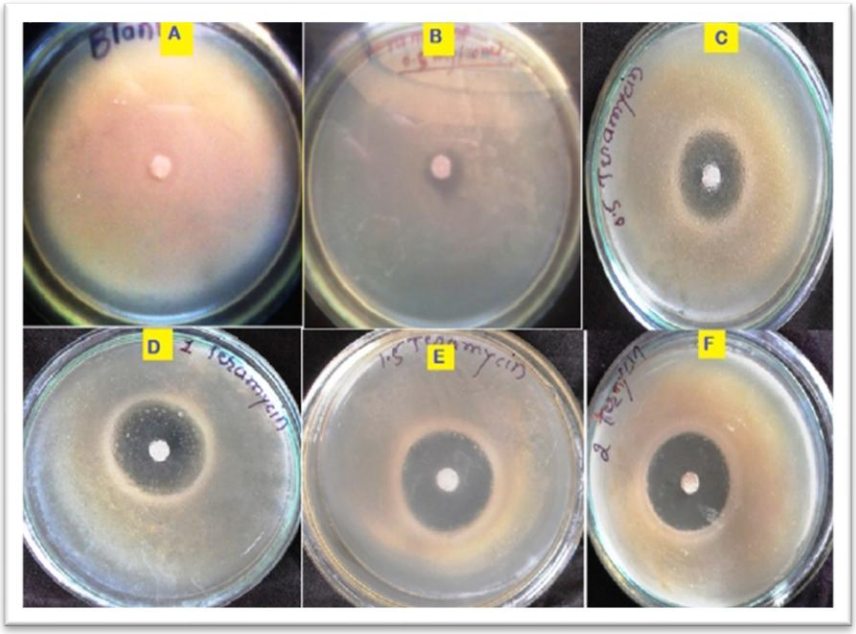

Fig. 8 Teramycine show zone of inhibition against *Xanthomonas axonopodis.*

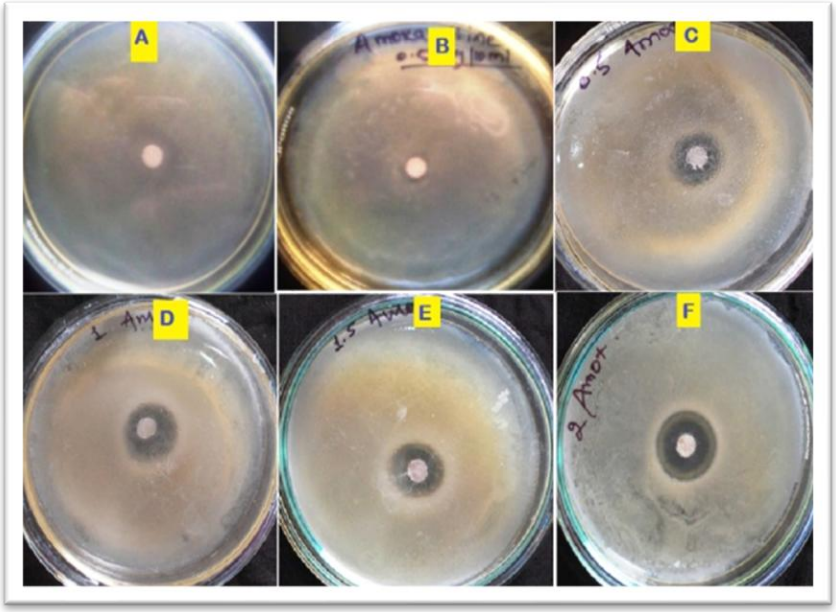

Fig. 9. Amoxicillin *antibiotics* show zone of inhibition against *Xanthomonas axonopodis*.

Table 1. Showing cultural characteristics of isolated microorganisms.

Sr. No.	Colony character	Result
1.	Size	2-3 mm upto 6 mm
2.	Pigmentation	Yellow
3.	Form	Circular
4.	Margin	Entire
5.	Elevation	Convex
6.	Consistency	Moist
7.	Surface	Smooth
8.	Gram staining	Gram negative
9.	Motility	Motile

Table 2. Showing biochemical test of microorganisms

Sr. No.	Biochemical test	Result
1	Indole test	-ve
2	Methyl red test	+ve
3	Vogues-Procures test	-ve
4	Citrate utilization test	+ve
5	Starch hydrolysis test	+ve
6	Casein hydrolysis test	+ve
7	Carbohydrate test a) Sucrose b) Lactose	No gas formation but acidic
8	Catalase test	+ve

Table 3. Showing activity of Biopesticide against *Xanthomonas axonopodis pv. citri*.

Sr.No.	Plant Extract	Concentration (ml/ml in Distilled Water)	Zone of Inhibition in (cm)
1	*Azadirachta indica*	0.1ml/ml	00
		0.2ml/ml	00
		0.5ml/ml	00
2	*Osmium santum*	0.1ml/ml	00
		0.2ml/ml	00
		0.5ml/ml	0.8

3	*Calotropis procera*	0.1ml/ml	0.9
		0.2ml/ml	1.2
		0.5ml/ml	1.4
4	*Chrysanthemum Cineriafolium*	0.1ml/ml	00
		0.2ml/ml	00
		0.5ml/ml	00
5	*Ipomea cornea*	0.1ml/ml	00
		0.2ml/ml	00
		0.5ml/ml	0.7
6	*Datura stramonium*	0.1ml/ml	0.8
		0.2ml/ml	1.0
		0.5ml/ml	1.1
7	*Zingiber officinale*	0.1ml/ml	00
		0.2ml/ml	00
		0.5ml/ml	00
8	*Curcuma longa*	0.1ml/ml	00
		0.2ml/ml	00
		0.5ml/ml	00
9	*Cathranthus roseus*	0.1ml/ml	00
		0.2ml/ml	00
		0.5ml/ml	00
10	*Nicotina tobacum*	0.1ml/ml	00
		0.2ml/ml	00
		0.5ml/ml	00
11	*Annona squamosa*	0.1ml/ml	00
		0.2ml/ml	00
		0.5ml/ml	00
12	*Aspergillus racimonas*	0.1ml/ml	00
		0.2ml/ml	00
		0.5ml/ml	00
13	*Argemone mexicana*	0.1ml/ml	1.8
		0.2ml/ml	1.9
		0.5ml/ml	2.3
14	*Jetropha sp.*	0.1ml/ml	00
		0.2ml/ml	00
		0.5ml/ml	00

Table 4 Antibiotic activity against *Xanthomonas axonopodis pv. citri*

Sr. no.	Antibiotic	Concentration (mg/ml in Distilled Water)	Zone of Inhibition (in cm)
1.	Tetracycline	0.05 mg/ml	1.0
		0.5 mg/ml	1.8
		1.0 mg/ml	2.1
		1.5 mg/ml	2.3
		2.0 mg/ml	2.6
2.	Ampicillin	0.05 mg/ml	00
		0.5 mg/ml	00
		1.0 mg/ml	1.4
		1.5 mg/ml	1.5
		2.0 mg/ml	1.5
3.	Amoxicillin	0.05 mg/ml	0.8
		0.5 mg/ml	1.3
		1.0 mg/ml	1.4

		1.5 mg/ml	1.4
		2.0 mg/ml	1.6
4.	Levofloxacin	0.05 mg/ml	1.6
		0.5 mg/ml	2.3
		1.0 mg/ml	2.7
		1.5 mg/ml	2.9
		2.0 mg/ml	3.0
5.	Ofloxacin	0.05 mg/ml	1.4
		0.5 mg/ml	2.1
		1.0 mg/ml	2.4
		1.5 mg/ml	2.6
		2.0 mg/ml	2.7
6.	Teramycine	0.05 mg/ml	1.4
		0.5 mg/ml	2.1
		1.0 mg/ml	2.4
		1.5 mg/ml	2.6
		2.0 mg/ml	2.7
7.	Cefixime	0.05 mg/ml	0.9
		0.5 mg/ml	1.6
		1.0 mg/ml	1.7
		1.5 mg/ml	1.9
		2.0 mg/ml	2.2
8.	Penicillin	0.05 mg/ml	0.7
		0.5 mg/ml	0.9
		1.0 mg/ml	1.2
		1.5 mg/ml	1.3
		2.0 mg/ml	1.5
9.	Azithromycin	0.05 mg/ml	00
		0.5 mg/ml	1.2
		1.0 mg/ml	1.4
		1.5 mg/ml	1.7
		2.0 mg/ml	2.0
10.	Cefadroxil	0.05 mg/ml	00
		0.5 mg/ml	00
		1.0 mg/ml	00
		1.5 mg/ml	00
		2.0 mg/ml	00