Research Article

Management of Citrus Canker Caused by *Xanthomonas axonopodis* pv. *citri* Using Essential Oils and Plant Extracts Under Laboratory Condition

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Abstract | Citrus fruits are the most common commercially grown fruit in the Rutaceae family worldwide. They are very nutritious and have a significant impact on the global economy. Its widespread cultivation has allowed it to rank first in fruit production in Pakistan. Unfortunately, several microbial diseases (like citrus canker caused by *Xanthomonas axonopodis pv. citri has* a great impact on the citrus industry. The current study was focused on checking the effectiveness of essential oils that contain antimicrobial chemicals that are less harmful, safe, eco-friendly, and readily biodegradable and assessed against *Xanthomonas axonopodis pv. citri* caused citrus canker. For the management of citrus canker, three essential oils (Neem, Clove, and Cinnamon oil) and Plant Extracts (Taiz Path and Garlic) were evaluated at different concentrations of 0.5, 1, and 2% in a lab setting. The maximum inhibitory effect was shown by Neem oil, followed by Clove oil and Cinnamon at 2% concentration, respectively. Among all tested concentrations, Clove oil showed the most prevention of citrus canker; 2% was the most effective concentration. These outcomes were consistent with earlier researchers emphasizing the powerful antibacterial qualities of Clove oil. Cinnamon oil also demonstrated inhibitory effects like clove oil but to a lesser extent. The outcome of this study is to encourage the use of essential oils as biological control and find out if plants show toxicity against pathogens. Since essential oils include a variety of antimicrobial components, we must use them to their fullest potential and assess how best to incorporate them into various integrated disease treatment methods. This tactic will also aid in lessening environmental risks and the harmful consequences they have on people.

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Introduction

Citrus fruits are the most common commercially grown fruit in the Rutaceae family worldwide. They are very nutritious and significantly impact the global economy (Russo *et al*., 2021). Citrus is grown on 13.9 million hectares worldwide, yielding 194.4 million tons of fruit annually. However, Pakistan grows 2.37 million tons of citrus on 206.6 thousand hectares (Aslam *et al*., 2024). According to Naqvi *et al*. (2022), The agriculture production of Pakistan is ranked 13th globally for citrus production, which spans 0.193 million hectares and yields 2.396 million tons. China, Brazil, and India are the three nations with the highest citrus production. Numerous issues during citrus farming potentially lower fruit quality and productivity. Pathogens like worms, bacteria, fungi, and viruses can also harm citrus fruit. This may impact the income of citrus growers and plant productivity (Khan *et al*., 2023). Different bacteria have been reported to cause diseases in citrus-growing orchards of Pakistan, including citrus canker, citrus gummosis, citrus decline, CTV, and HLB (Mubeen *et al*., 2024a, b; Iftikhar *et al*., 2024). Canker disease poses a major danger to citrus farming worldwide, resulting in significant annual losses (Mubeen *et al*., 2015a, b). This disease is mostly caused by the Xcc bacterium, which occurs in many strains and can significantly reduce the nutritional and market value of citrus fruits. The disease is caused by *Xanthomonas axonopodis pv. citri* globally (Gottwald and Irey, 2007). The gram-negative, rod-shaped citrus canker bacterium has a single polar flagellum. The bacterium needs an aerobic environment that is between 35 to 39 degrees Celsius in order to grow. Small to medium-sized yellow colonies grew in the convex and mucoid culture conditions (Gupta, 2016). In 1910, the first signs of citrus canker were discovered in the United States, close to the border between Georgia and Florida. Asia was originally documented in the Indian state of Punjab (Luthra and Sattar, 1942). Since then, reports on its incidence have been released by all states that cultivate citrus (Das, 2003). Lesions of varying sizes that initially appeared as pinpoints on the leaf surface, particularly on the axial side, are the hallmarks of citrus bacterial canker disease. The lesions develop a depressed center and elevated edge surrounded by a yellow halo after a few days, giving them a corky appearance. Fruit lesions vary in size, and because fruit surfaces may hold many infection cycles, fruits are more prone to infection than leaf surfaces (Derso *et al*., 2007). Early fruit loss, twig dieback, defoliation, and fruit blemish can all indicate the severity of the disease. According to Dewdney and Graham (2016), too early or unmarketable fruits result in the most financial losses. The most common tests used in pathogen detection serological methods are physiological and biochemical. It must be cultivated on various media to detect the pathogen using pathogen detection techniques, which takes time and involves multiple processes. Consequently, molecular biology-based techniques like the polymerase chain reaction (PCR), which uses several genomic regions, are used to find Xac (Abubakar *et al*., 2016). Moreover, Xanthomonas is quickly identified and detected by PCR techniques, which are more precise and faster than previous approaches. However, this method has certain drawbacks, such as needing an expensive heat cycler fitted with a premium fluorescence detector (Mavrodieva *et al*., 2004). Traditionally, the disease has been eradicated, windbreaks have been constructed, copper-based bactericides have been applied, and the affected plants have been trimmed. However, the primary method of combating citrus canker disease is the creation of resistant types (Gottwald *et al*., 2002). Applying copper-based pesticides as soon as possible is the main strategy to stop the spread of disease (Behlau *et al*., 2008). Diseased trees should be trimmed and topically treated with copper bactericides to reduce disease risk. The disease incidence was also reduced by using essential oils, plant extracts, and other drugs. In theory, these disease control techniques may reduce the risk of disease severity and death of the trees. However, they are costly and raise the possibility of soil and air pollution due to their widespread application of copper chemical sprays. However, the pathogens resist continuously applying copper compounds (Rinaldi and Leite, 2000). Approximately 17,500 plant species belonging to various plant families like the *Rutaceae, Zingiberaceae, Myrtaceae, Asteraceae*, and *Lamiaceae*, produce essential oils (EOs), which are hydrophobic, naturally occurring compounds (Merillon and Rivière, 2018). They can be found in the bark, flowers, leaves, roots, twigs, and seeds, among other parts of the plant (Bakkali *et al*., 2008). There are many ways to extract essential oils from different plant parts. However, steaming hydro-distillation is the most common method to extract essential oils (Nazzaro *et al*., 2017). EOs are incredibly complex substances that contain anything from 20 to 60 components in varying amounts. According to

Raveau *et al.* (2020), essential oils typically consist of one or two major components at concentrations ranging from 20% to 95% and one or more minor components at deficient concentrations. Citrus peel oils, for example, have more than 80% d-limonene, but *Origanum compactum* oil has high levels of thymol and carvacrol roughly 27% and 30%, respectively in its oil (Chouhan *et al.*, 2017). The chemical composition of essential oils (EOs) can vary depending on several factors, including the type of plant, the portion of the plant that is extracted, the age of the plant, the location, the climate, the composition of the soil, the cultivation methods employed, and the extraction process (Perczak *et al.*, 2019). Amini *et al.* (2018) found that applying *Z. multiflora* EO at a concentration of 232 µg/mL completely inhibited the growth of *Xanthomonas campestris* pv. *campestris* strains. This study uses environmentally friendly products like essential oils and plant extracts to manage citrus canker disease. The study will be carried out to meet the following objectives: To check the efficacy of essential oils and plant extracts against *X. axonopodis*.

Materials and Methods

Sample collection

The plant was examined closely, and the signs of citrus canker were on the affected leaves of the plants (Figure 1). The samples were collected and stored in a plastic/ zipper bag for experimentation. After that, they were stored in the lab, where the bacteria were isolated and refrigerated at 4°C for the supposed research.

Media preparation and isolation

The pathogen was isolated and purified using a nutritional agar medium according to the procedure described by Mubeen *et al.* (2015a, b). A one-liter sterile beaker was filled with fourteen grams of nutritious agar and 5 mL of distilled water. The media was autoclaved at 121°C and 15 pressure to sterilize it. A tiny piece of healthy leaf was carefully removed from the damaged area. 0.1% $HgCl_2$, distilled water, and distilled water were used to sterilize the surface. The samples were placed on blotter paper to eliminate moisture after surface sterilization. In order to protect the samples and the experiment's integrity, the samples were placed on poured plates that contained NA medium and covered with packing tape. The plates were then labeled appropriately and incubated for twenty-four hours at 28°C.

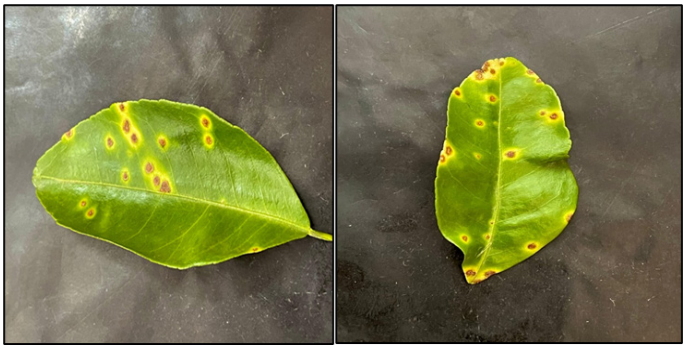

Figure 1: *Canker symptoms observed on grapefruit leaves.*

Purification and multiplication

The streaking method was used to purify the bacteria after incubating them for 24 hours. After isolating a single colony of bacteria, it was grown at 28°C and then reproduced on plates for purification. The morphology and biochemical characteristics of bacterium allow for identification. The stock culture was kept in a refrigerator at 4°C.

Pathogenicity test

The pathogenicity of the isolated bacterium was tested on healthy citrus plants (Mayer grapefruit and lemon) obtained from the University of Sargodha's Horticulture Nursery. These plants were transferred to pots with soil sterilized using 5% formalin. The bacteria were cultured for 48 hours at 30°C±2°C on nutrient agar, and a solution with a concentration of ~108 cells/ml was prepared using the plate count method. After watering the plants, they were inoculated, covered with polythene to maintain high humidity, and exposed to sunlight to open stomata. The abaxial leaf surface was sprayed with the infected solution until signs of water soaking appeared, while control plants were sprayed with sterilized water. The plants were observed in the greenhouse for two weeks, and symptoms were recorded. Bacteria were re-isolated from diseased tissue and compared to the original culture to confirm pathogenicity.

In-vitro evaluation of essential oils against Xanthomonas citri pv. citri through inhibition zone technique

Citrus canker was managed by using essential oils and plant extracts. The oil extracts that were used in the experiments were neem oil (*Azadirachta indica*), clove oil (*Syzygium aromaticum*), and cinnamon oil (*Cinnamomum zeylanicum*). However, Plant extracts used in experiments are Taiz Path (*Cinnamomum tamal*) and Garlic (*Allium sativum*). Each oil and plant extract was utilized by average concentrations of 0.5%, 1%, 1.5%, and 2% (Table 1). Add 0.5 ml to

99.5 ml, 1 ml to 99 ml, and 2 ml to 98 ml of molten Nutrient Agar to make the oils. However, fresh plant material leaves, rhizomes, bulbs, and branches were gathered to make plant extracts. A blender was used to macerate 75 g of fresh material individually with 25 ml of sterilized distilled water to prepare aqueous extracts from the abovementioned plant. The macerates were filtered through Whatman filter paper no. 4 after initially passing through four layers of sterilized muslin cloth. The extracted material obtained from this process was deemed standard (S) arbitrary by (Ilyas *et al*., 1997) and was kept in a deep freezer for cold sterilization and further laboratory research. According to (Ilyas *et al*., 1997), the media was solidified, and a 5 mm aseptic bacterial plug of *X. axonopodis* was inserted into the center of the NA plate using a sterile cork borer. After covering the plate with parafilm, these were incubated at 25±10 C. There were three copies of each concentration. As a control, NA petri plates devoid of essential oils were used. Using a scale, measurements of the bacterial growth were made on both sides of the perpendicular lines drawn beneath the Petri plates. After that, the replication means were added and divided by three to get the average mean. The data was collected after a total of 168 hrs. The following formula will calculate the data (Skidmore and Dickinson, 1976).

$$\textit{Radial growth inhibition \%} = R_1 - R_2 / R_1 \, X \, 100$$

Table 1: *List of Essential oils concentrations used in laboratory.*

Treatments	Common name	Scientific name	Concentrations %
T_1	Cinnamon	*Cinnamomum zeylanicum*	0.5,1,2
T_2	Clove	*Syzygium aromaticum*	0.5,1,2
T_3	Neem	*Azadirachta indica*	0.5,1,2
T_2	Taiz Path	*Cinnamomum tamal*	0.5,1,1.5
T_3	Garlic	*Allium sativum*	0.5,1,1.5

Statistical analysis

The data from different experiments will be analyzed using variance analysis (ANOVA) and LSD using SPSS software.

Results and Discussion

Pure culture of Xanthomonas axonopodis

The pathogen was isolated using an NA medium. *Xanthomonas axonopodis* were identified based on their morphological characteristics (Figure 2).

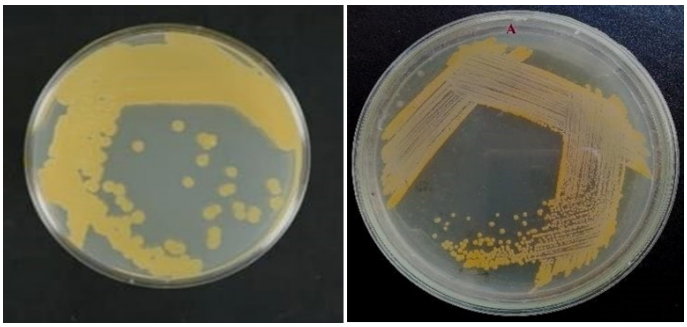

Figure 2: *Pure culture of Xanthomonas axonopodis.*

KOH test

To perform the test, bacteria are aseptically removed from the agar medium, placed on a glass slide in a drop of 3% KOH test, and stirred for 10 sec using a quick circular motion (Figure 3).

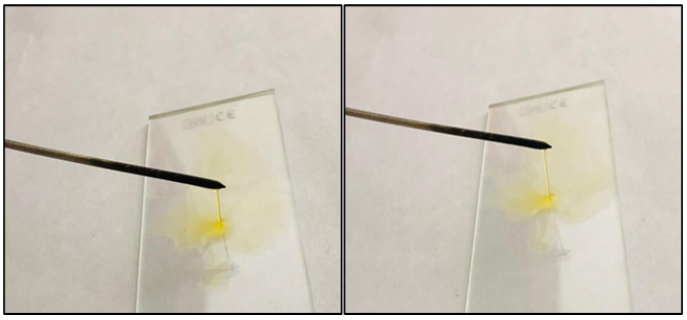

Figure 3: *Biochemical identification of Xanthomonas axonopodis.*

Bacterial growth of Xanthomonas axonopodis treated with essential oils

The bacterial growth of *Xanthomonas axonopodis* treated with three different essential oils (Cinnamon, clove, and neem oil) was calculated and recorded, and findings of the study revealed non-significant differences among treatments compared to control and given treatments were contributing to the development of the bacterial growth.

Effect of different treatments on bacterial growth

The results revealed that treatments differed significantly from each other and compared to the control group. The neem oil reduced the maximum mycelial growth with a mean value of 0.87cm, followed by cinnamon at 1.38 cm and clove at 1.69 cm, whereas the mean value of control was 1.87cm. This information showed that neem effectively controls bacterial growth, with the highest growth in control. Graphical representation demonstrated that the lowest mycelial growth was shown by neem oil, while clove oil showed the highest growth as compared to other treatments (Figure 4).

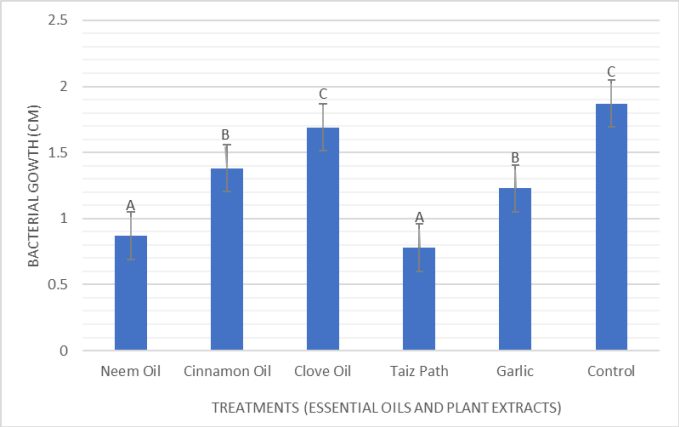

Figure 4: *Effect of different treatments on growth of Xanthomonas axonopodis.*

Effect of different concentrations on bacterial growth
The results showed that concentrations were significantly different from each other. The 0.5% concentration reduced the maximum mycelial growth with a mean value of 1.02cm, followed by 1%, 1.38cm, and 2%, 1.96cm. This information showed that a 0.5% concentration is much more effective in controlling mycelial growth, with the highest growth at 2%. Graphical representation demonstrated that the 0.5% concentration showed the lowest mycelial growth, while 2% showed the highest growth compared to other concentrations (Figure 5).

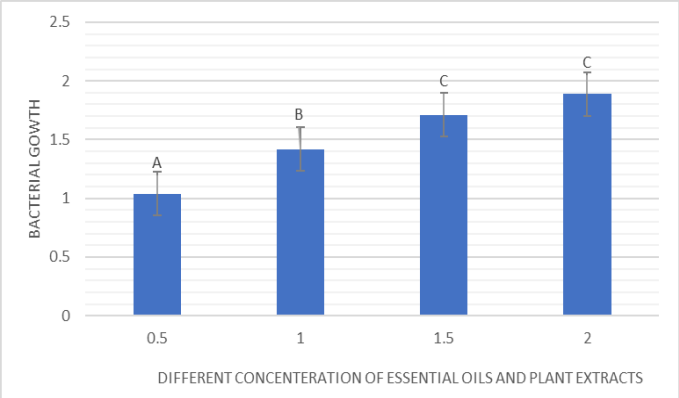

Figure 5: *Effect of different concentrations on the growth of Xanthomonas axonopodis.*

Comparison of means of concentration x treatment
The mean value showed three concentrations of cinnamon oil at 0.5%, which was 0.78cm, 1% was 1.22cm, and 2% was 2.13cm. The mean value showed four Neem oil concentrations: 0.5% was 1.23cm, 1% was 1.70cm, and 2% was 2.15cm. The mean value showed four control concentrations: 0.5% was 1.48cm, 1% was 1.86cm, and 2% was 2.26cm. The mean value showed three concentrations of neem oil at 0.5%, which was 0.68cm, 1% was 0.92cm, and 2% was 1.02cm. All the results concluded that Neem oil showed the lowest growth at 0.5%, while Clove oil showed the highest growth at 2%. The main effect was highly significant, while their associated interactions were significant, except treatment x days, Concentration x days, and treatment x concentration x days showed a significant response. Graphical representation explicitly demonstrated that neem oil showed the lowest bacterial growth in vitro at 2% concentration, while clove oil showed the highest growth at the same concentration as the control (Figure 6).

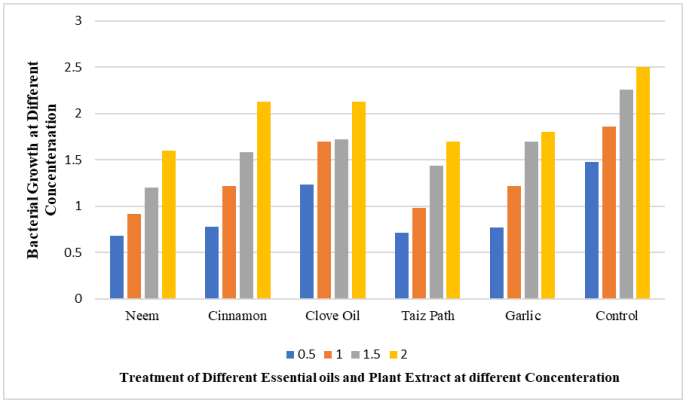

Figure 6: *In vitro efficacy of different essential oils and plant extracts against Xanthomonas axonopodis at different concentrations.*

Radial growth inhibition
The radial growth inhibition of *Xanthomonas axonopodis* treated with three different oils (cinnamon, clove, and neem oil) was calculated and recorded. The study's findings revealed non-significant differences among treatments compared to the control, and the given treatments contributed to inhibiting radial growth.

Treatment comparison in radial growth
The results showed that treatments were significantly different with each other. The neem oil reduced the maximum radial growth with a mean value of 56.85%, followed by cinnamon oil at 64.62 % and clove at 65.37 %. This information showed that neem effectively controlled radial growth, with the highest growth in clove. Graphical representation demonstrated that the lowest radial growth was shown by neem oil, while clove oil showed the highest growth as compared to other treatments (Figure 7).

Effect of different concentration on radial growth
The results revealed that concentrations were significantly different from each other. The 0.5% concentration reduced the maximum bacterial growth with the mean value of 54.74%, followed by 1% at

62.48 % and 2% at 69.62%. This information showed that a 0.5% concentration is much more effective in controlling bacterial growth, and the highest growth was at 2 %. This information showed that 0.5% concentration controls much radial growth while 2% concentration shows the highest growth. Graphical representation demonstrated that the lowest radial growth was by 0.5% concentration, while 2% showed the highest growth compared to other concentrations (Figure 8).

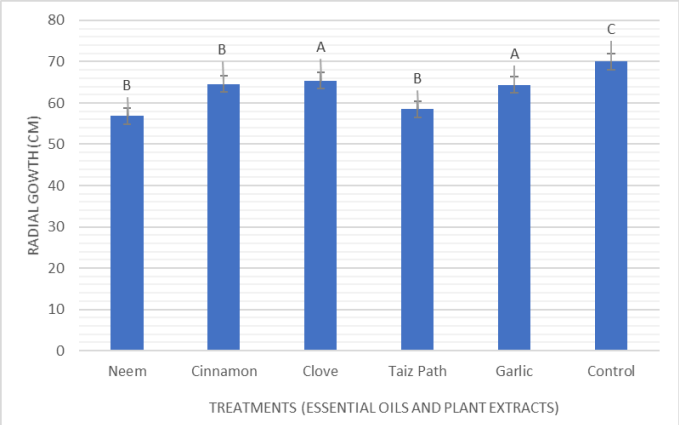

Figure 7: *Effect of different treatments on radial growth of Xanthomonas axonopodis.*

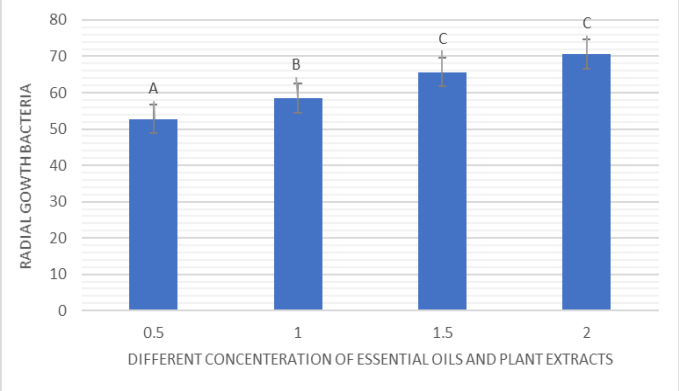

Figure 8: *Effect of different concentrations on radial growth of Xanthomonas axonopodis.*

Efficacy of different essential oils and plant extracts against Xanthomonas axonopodis at different concentrations

The mean value showed three concentrations of cinnamon oil at 0.5%, which was 59.66%, 1% was 63.22%, and 2% was 71.00%. The mean value showed three concentrations of clove oil at 0.5%, which was 58.77%, 1% was 64.44%, and 2% was 72.88%. The mean value showed three concentrations of neem at 0.5%, which was 45.77%, 1% was 59.77%, and 2% was 65.00%. All these results concluded that neem showed the lowest growth at 0.5% concentration while the highest growth is showed by clove oil at 2% concentration. The main effect was highly significant, while their associated interactions were significant, except concentration x days showed a non-significant response (Table 8). Graphical representation demonstrated explicitly that neem oil showed the lowest radial growth invitro at 2% concentration while cinnamon and clove oil showed the highest growth at the same concentration (Figure 9).

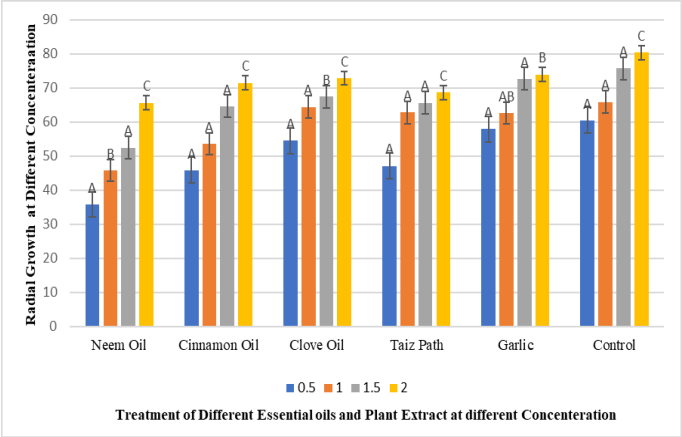

Figure 9: *In vitro efficacy of different essential oils and plant extracts against Xanthomonas axonopodis at different concentrations.*

Pathogenicity

The pathogenicity of *Xanthomonas axonopodis* treated with three different oils (Cinnamon, clove, and neem oil) was calculated and recorded. The study's findings revealed non-significant differences among treatments compared to the control, and given treatments contributed to the disease incidence.

Effect of different treatments on pathogenicity of Xanthomonas axonopodis

The results showed that treatments were significantly different with each other. The neem oil reduced the maximum disease incidence with a mean value of 26.03%, followed by cinnamon at 33.77% and clove oil at 37.44%. This information showed that neem oil was much more effective in controlling disease incidence, and the highest incidence was in clove oil. Graphical representation demonstrated that the lowest disease incidence was shown by neem oil, while clove oil showed the highest growth as compared to other treatments (Figure 10).

Effect of different concentration on pathogenicity of Xanthomonas axonopodis

The results revealed that concentrations were significantly different from each other. The 0.5% concentration reduced the maximum disease incidence with a mean value of 30.29%, followed by 1%, which was 32.81%, and 2%, which was 34.14%.

These results showed that the disease incidence was much controlled at 0.5% concentration, while the 2% concentration showed the highest incidence. Graphical representation demonstrated that the lowest disease incidence was a 0.5% concentration, while 2% showed the highest growth compared to other concentrations (Figure 11).

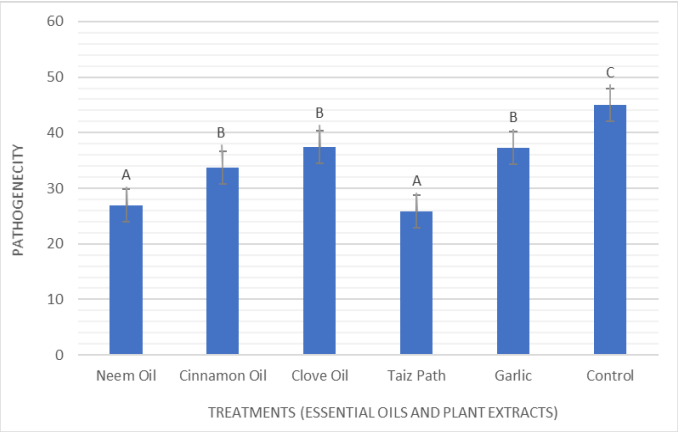

Figure 10: *Effect of different treatments on pathogenicity.*

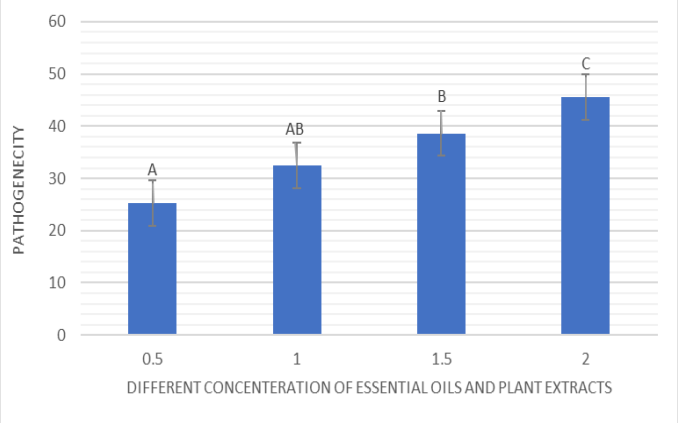

Figure 11: *Effect of different concentrations on pathogenicity.*

Efficacy of different essential oils against Xanthomonas axonopodis at different concentrations

The mean value showed three concentrations of Cinnamon oil at 0.5%, which was 31.33%, 1% was 34.00%, and 2% was 36.00%. The mean value showed three concentrations of three concentrations of Clove oil at 0.5%, which was 34.44%, 1% was 38.77%, and 2% was 39.11%. The mean value showed three concentrations of neem oil as 0.5% was 25.11%, 1% was 25.33%, and 2% was 27.66%. These results concluded that neem oil showed the lowest disease incidence at 0.5% concentration, while the highest incidence showed clove oil at 2% concentration. The main effect was highly significant, while their associated interactions were significant. Treatment x concentration, concentration x days, and treatment x concentration x days showed a non-significant response. Graphical representation explicitly demonstrated that neem oil showed the lowest disease incidence in vitro at 2% concentration, while clove oil showed the highest at the same concentration (Figure 12).

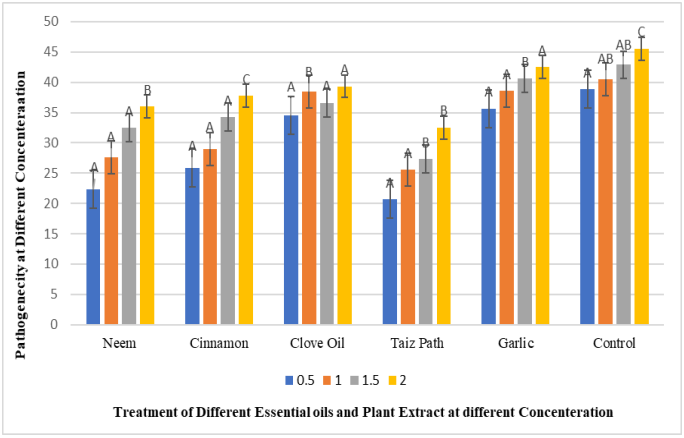

Figure 12: *In vitro efficacy of different essential oils against Xanthomonas axonopodis at different concentrations.*

Citrus is a genus from the *Rutaceae* family comprised of citrus fruits. Citrus is the second-fastest-growing fruit with the largest worldwide market (Lee *et al.*, 2019). Although efficient, using synthetic chemicals has negative consequences for the environment. Chemical use over time leads to the development of pathogen resistance (Waqas *et al.*, 2018). Because essential oils contain antimicrobial chemicals that are less harmful, safe, eco-friendly, and readily biodegradable. These essential oils and Plant extracts were assessed in the current study about citrus cankers (Rezaei *et al.*, 2020). Cinnamon oil (*Cinnamomum zeylanicum)*, Clove oil (*Syzyzium aromaticum)*, and Neem oil (*Azadirachta indica*) are three essential oils that were tested in a lab setting against *Xanthomonas axonopodis,* the causative agent of citrus canker. Clove oil exhibited the largest inhibition zone at all concentrations and was the most effective in the lab setting. The search for more ecologically friendly ways to treat plant diseases has accelerated. Because of growing consumer concerns and chemical residues in soils and waterways, researchers are searching for more ecological ways to handle phytopathogens in the field. Some recent studies have suppressed citrus cankers using the antagonistic action of microbes and chemicals produced by plants (Martin *et al.*, 2019). As pathogens' resistance is increasing daily against different pesticides (Atiq *et al.*, 2018; Waqas *et al.*, 2018), the world is coming to natural bio-stimulants to control the different types of pathogens. Our finding is also similar to the result of (Najam *et al.*,

2012), who assessed the essential oils of *Xanthomonas* species, which are phytopathogenic bacteria, and pure constituents like limonene, oc-terpineol, p-cymene, eugenol, and linalool. The essential oils were evaluated from *Pimpinella anesum, Artemisia annua, Cymbopogon martini, Mentha piperita,* and *Apium graveolens*. The greatest growth inhibition against *Xanthomonas citri* demonstrated in vitro by pure ingredients such as a-terpineol and eugenol, as well as essential oils of *Apium graveolens*, was 84, 86, and 84%, respectively. Additionally, they demonstrated that the growth of *X. citri* may be suppressed by essential oils from Cymbopogon martini and p-cymene by up to 81 and 53%, respectively. According to the study, the pure constituents a-terpineol and eugenol, as well as the studied extracts from A graveolens, may naturally suppress the growth of *Xanthomonas* species. Antimicrobial properties of some essential oils affect the growth of *Xanthomonas citri* subsp. *citri*. The main objective was to provide a green substitute for synthetic chemicals by testing the finest essential oils as plant byproducts to manage crop diseases. Clove oil (*Syzyzium aromaticum* and Cinnamon oil (*Cinnamomum zeylanicum)* showed good antibacterial activity (Rezaei *et al*., 2020). Current studies revealed that the essential oils and chemical fungicides were highly effective against citrus canker. Therefore, detailed studies should be directed toward the in-vitro management of Citrus canker with the help of essential oils. In most cases, bacterial growth progressively decreased as the dosage of essential oils increased. Among the used extracts, the neem extract was more effective in inhibiting the colony growth of *X.axonopodis*. The first essential oil used for the Citrus canker control was Cinnamon oil (*Cinnamomum zeylanicum)*, with a concentration of 0.5%, 1%, and 2% in the lab conditions. At the concentration of 2%, there was more inhibition in the *Xac* growth than in the other concentrations. It was confirmed by testing the three replications, and from all, it was observed that a 2% concentration of the cinnamon oil was more favorable for inhibiting the Xac growth on citrus plants. It also confirms the findings of (Najam *et al*., 2012), who checked the essential oils at different concentrations in the media plates and observed that essential oils naturally suppress the growth of *Xanthomonas* species. Our findings also confirm that as the concentration of the cinnamon oil increased and then sprayed on the canker growing areas, it inhibited canker growth. Although the other concentrations also inhibit the citrus canker growth, the 2% shows the major impact, as shown by the 0.5% and 1% concentrations. The second essential oil used for the citrus canker control was Clove oil (*Syzyzium aromaticum)*, with concentrations of 0.5%, 1%, and 2% in the lab conditions. At the concentration of 2%, there was more inhibition in the Canker growth than in the other concentrations. It was confirmed by testing the three replications, and from all, it was observed that a 2% concentration of the clove oil was more favorable for inhibiting the Xac growth on citrus plants. It also confirms the findings of (Rezaei *et al*., 2020), who checked the essential oils at different concentrations in the media plates and observed that essential oils naturally suppress the growth of *Xanthomonas* species. Our findings also confirm that as the concentration of the clove oil increased and then sprayed on the canker growing areas, it inhibited canker growth. Although the other concentrations also inhibit the citrus canker growth, the 2% shows the major impact, as shown by the 0.5% and 1% concentrations. The third essential oil used for the Fusarium wilt of okra control was Neem oil (*Azadirachta indica*), with concentrations of 0.5%, 1%, and 2% in the lab conditions. At the concentration of 2%, there was more inhibition in the Xac growth than in the other concentrations. It also confirms the findings of (Marin *et al*., 2024), who checked the same concentration in the media plates and observed the maximum inhibition at the concentration of 2%. Our findings also confirm that as the concentration of the neem oil increased and then sprayed on the canker growing areas, it inhibited canker growth. Although the other concentrations also inhibit the citrus canker growth, the 2% shows the major impact, as shown by the 0.5% and 1% concentrations.

Conclusions and Recommendations

The citrus sector in Pakistan faces a significant danger from citrus canker, which impacts all commercial kinds and areas. Although synthetic chemicals are the most effective way to prevent canker disease, there is a need for environmentally acceptable alternatives because chemicals have side effects. Based on the current findings, it can be said that essential oils with antibacterial properties are being researched as environmentally friendly alternatives to deal with this issue. Clove oil was discovered to be the most successful essential oil in managing citrus canker. Since essential oils include a variety of antimicrobial components, we must use them to their fullest potential and assess how best to incorporate them into various integrated

disease treatment methods. This tactic will also aid in lessening environmental risks and the harmful consequences they have on people.

Novelty Statement

Essential oils and plant extracts will help in developing eco-friendly management of citrus canker.

Author's Contribution

Yasir Iftikhar: Conceptualized and supervised the trial.
Talha Shafique: Statistical analysis and writing the original draft.
Shahzaib Asghar and Maham Ikram: Conducted the research trial.
Sonum Bashir, Farwa Seemab, Komal Ambreen, and Ashara Sajid: Helped with fieldwork and technical assistance for lab analysis.
Muhammad Ahmad Zeeshan and Malik Abdul Rehman: Co-supervised the trial.

Conflict of interest
The authors have declared no conflict of interest.

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