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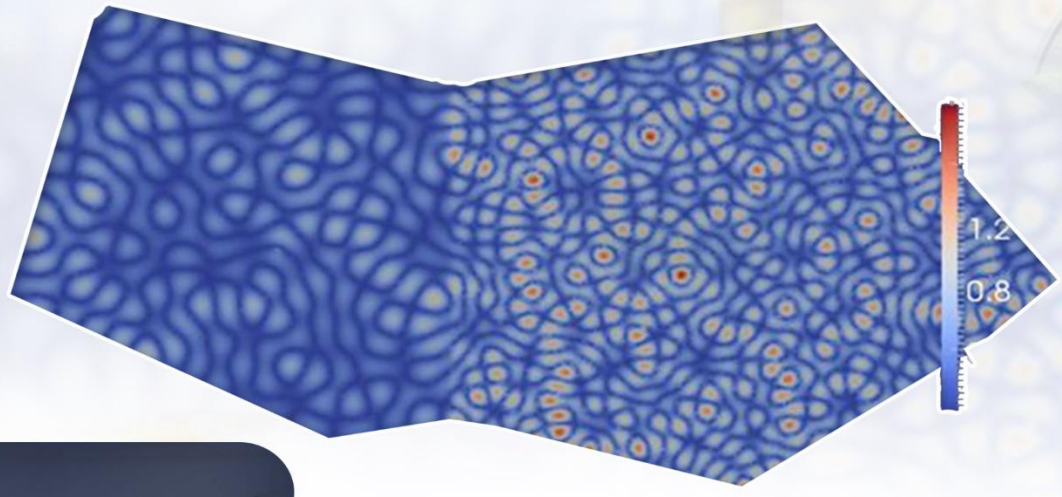

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Antibacterial Activity of *Cyclamen rohlfsianum* against two Species of *Xanthomonas*

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A B S T R A C T

Xanthomonas is one of the most important genera that leads to severe losses in many agricultural crops, the exertion to control is constrained to the utilisation of industrial pesticides, which have brought human and environmental health problems, and the emergence of resistant strains. This work was carried out to evaluate the activity of the aqueous extracts of leaves and tubers of *Cyclamen rohlfsianum* at concentrations (50, 100, 200 and 400) mg/ml against *(Xanthomonas campestris* pv. *vesicatoria* and *Xanthomonas axonopodis* pv. *eucalyptus)* by a sensitivity test by a disk diffusion method. The results showed that *Cyclamen rohlfsianum* extracts have good inhibitory activity against tested bacteria, the tubers extracts are more efficient than leaf extracts, a concentration of 400mg/ml was the most effective to control bacteria. *Xanthomonas axonopodis* pv. *eucalyptus* was the most sensitive to aqueous extracts. The study concluded that *Cyclamen rohlfsianum* showed promising antibacterial activity and effective control programs for the control of diseases caused by *Xanthomonas.*

1 Introduction

Plants are influenced by numerous illnesses caused by organisms, the predominance of illnesses can reach 70-80%. Depending on climate conditions and the phytosanitary condition of crops (Nazarov *et al*., 2020). Bacterial plant diseases are most severe in tropical and subtropical places, it's recorded in all countries agricultural crop high losses, especially in developing countries (Campos and Ortiz, 2020), from common symptoms of bacterial plant diseases are wilting, necrosis, spotting, rotting, blight, blisters (El-Meneisy *et al*., 2005). *Xanthomonas* are a large genus of Gram-negative bacteria, yellow-pigmented, rod-shaped, a single polar flagellum (Bradbury, 1984). The genus consists of 27 species that cause serious diseases in almost 400 plants including a wide variety of important crops such as rice, citrus, cabbage and pepper, including *Xanthomonas campestris* pv. *vesicatoria* and *Xanthomonas axonopodis* pv. *eucalyptus*, which causes diseases to tomato and eucalyptus (Ryan *et al*., 2011; Ferraz *et al*., 2018), Measures to combat it are limited to the use of antibiotics and industrial pesticides, especially those made up of copper compounds, due to their harmful side effects, the emergence of resistant strains, the trend has been to use natural alternatives with low environmental impact represented by medicinal plants (Gochez *et al*., 2018; Puigvert *et al*., 2019). *Cyclamen spp*. a small genus consisting of 21 species distributed in the Mediterranean region, Europe, Western Asia and North Africa. used as an ornamental plant in gardens (Yesson *et al*., 2009), a potential source of natural antioxidants and antimicrobial (Stanojevic *et al*., 2018). *Cyclamen rohlfsianum* is an endemic strain, only grows in Al-jabal Al-akhdar region, Libya, known locally as Al-Rakhf, a perennial tuberous herbaceous plant, belonging to the family Primulaceae (Salih and Abdulrraziq, 2021), It's classified as a poisonous plant because it contains Cyclamen glycoside (Chant, 1979), the plant has also been reported to show pharmacological properties to the therapy of diabetes, Its tubers are used in the process of fermenting milk to produce cheese (Elabbar *et al*., 2014). Several studies

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have been conducted in an attempt to combat various diseases caused by *Xanthomonas spp.* using plant extracts, where a study concluded in Libya an extract of leaves of *Arum cyreniacum* can be relied on to control of *X.campestris* pv.*vesicatoria* which cause tomato spots disease (Abdulrraziq *et al*., 2021). Furthermore, findings from a study that was conducted in India confirmed the effectiveness of alcoholic extracts of six types of medicinal plants in eliminating black rot of cauliflower caused by *Xanthomonas campestris* pv. *campestris* (Didwania *et al*., 2013). The results of a study conducted in Korea showed of a methanolic extract of *Pharbitis nil* seeds possesses a high inhibitory ability against *Xanthomonas aboricola* pv. *pruni*, that causing bacterial spot disease of peach (Nguyen *et al*., 2017). The results of another study indicated the possibility thyme oil and lemon grass oil effectively retarded pomegranate bacterial blight disease caused by *Xanthomonas axonopodis* pv. *punicae* (Chowdappa *et al*., 2018).

Therefore, the study aimed to evaluate the efficacy of aqueous extracts of *Cyclamen rohlfsianum* to bio-control against two species *Xanthomonas*, which had never hitherto been established.

2 Materials and Methods

The study was carried out in Biology Department/ Faculty of education / Omar Al-Mukhtar University. Plant washing (leaves- Tubers) with distilled water and dried inside the laboratory under room temperature, grinded by an electric grinder and saved for use.

Figure (1): *Cyclamen rohlfsianum.*

2.1. Aqueous Extraction:

10 g of dry powder of the plant was added to 100 ml of sterile distilled water in a glass flask, put on a vibratory shaker for 24 hours at 35 ° C, then filter and shaken in a centrifuge at 3000 rpm for 10 minutes. The next step was filtered with Whitman No.1 filter paper and drying in a Rotary evaporator to get dry powder (Jigna *et al*., 2005). The concentration of 400 mg/ml was prepared by dissolving 4g of powder in 10 ml distilled water.

2.2. Bacterial Isolates:

Xanthomonas campestris pv. *vesicatoria* and *Xanthomonas axonopodis* pv. *eucalyptus*, from the collection of the Department of Plant Protection, Omar Al-Mukhtar University.

2.3. Antibacterial Susceptibility Testing:

The mediums were sterilized for 15 minutes in an autoclave at 121°C. bacteria were grown on Mueller-Hinton agar medium. For screening, sterilised filter paper disks (6 mm) and impregnated with the extract were placed on the surface of inoculated bacteria mediums. The dishes were incubated for 24 hours at 28°C with three replications per dish, With the use distilled water to control, then a measure of diameters of inhibitory zones minus the diameter of the disc (Driscoll *et al*., 2012).

Statistical Analysis:

The study's experiment was designed according to the Completely Randomized Design (CRD). The statistical analysis was performed using (Minitab 17) program and ANOVA test was carried out means were compared using Tukey's test at $P<0.05$.

3 Results

The results of antibacterial activity were recorded as a zone of inhibition in mm around the extract disk against the tested bacteria compared with the control. As shown in table(1), there were significant differences in the inhibitory activity of extracts of leaves and tubers of *Cyclamen rohlfsianum* against plant pathogenic bacteria, according to the type of microbe, concentration and type of part used. The concentration of 200 and 400 mg/ml of the tubers extract recorded good inhibitory activity against two species *Xanthomonas* with a diameter ranging (3.1-4.4) mm. The results also showed that the Tubers extract with a concentration of 50 and100 mg/ml had no inhibitory effect against the tested bacteria, except for a concentration of 100mg/ml against *X.axonopodis* pv. *eucalyptus* with a diameter (2.9) mm. The results showed that the leaves extract with a concentration of 50,100 and 200mg/ml had no inhibitory effect against the tested bacteria, except for a concentration of 200mg/ml against *X.axonopodis* pv. *eucalyptus*, which showed low sensitivity with a

diameter (1.4) mm, while both types of *Xanthomonas* were affected by the concentration of 400mg/ml with a diameter of inhibition that did not exceed (2.0) mm.

Table (1). Antibacterial activity of *Cyclamen rohlfsianum* aqueous extracts agints *Xanthomonas*. (Mean ± Standard Deviation).

Extract \ Bacteria		*X. campestris pv. vesicatoria* (mm)	*X. axonopodis pv. eucalyptus* (mm)
Leaves	**50 mg/l**	0.0 d	0.0 d
	100	0.0 d	0.0 d
	200	0.0 d	1.4±0.5 c
	400	1.2±0.1 c	2.0±0.3 c
Tubers	**50 mg/l**	0.0 d	0.0 d
	100	0.0 d	2.9±0.3 b
	200	3.1±0.3 b	3.6±0.4 ab
	400	3.5±0.2 a	4.4±0.3 a
Control		0.0 d	0.0 d

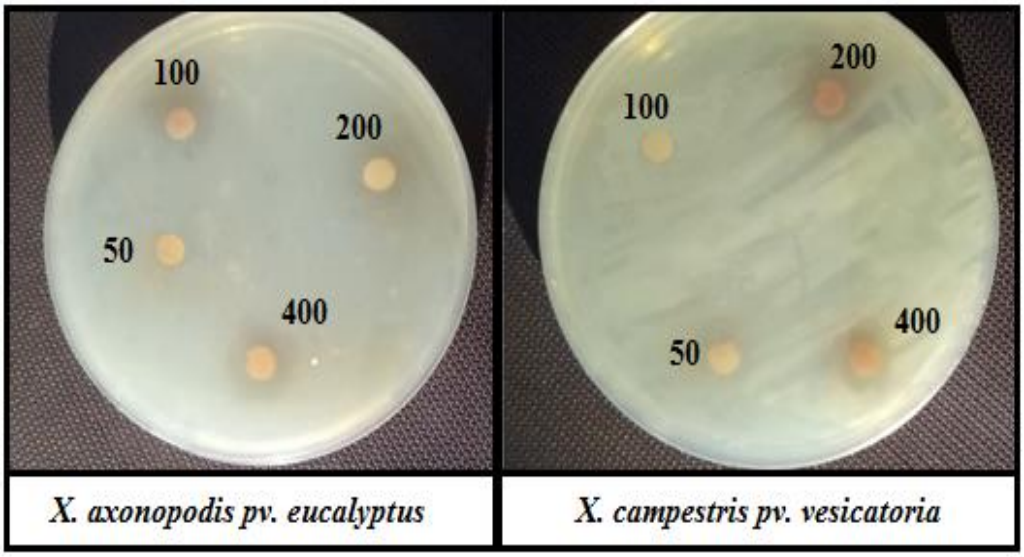

Figure (2): Effect of tubers extract of *Cyclamen rohlfsianum* against *Xanthomonas***.**

4 Discussion

Al-Jabal Al-Akhdar is considered as one of the most important Libyan regions in terms of its diversity of native medicinal plants, which it does not obtain a received of full studies Bio-activities (Agile and Mericli, 2017), So this study was conducted to which showed of *Cyclamen rohlfsianum* extracts possess good inhibitory activity against two species of Xanthomonas, This result was in agreement with (Yemata and Fetene, 2017; Abo-Elyousr *et al*., 2020 ; Leksomboon *et al*., 2001), where they confirmed the medicinal plants have promising antibacterial activity, and could be considered an effective tool in integrated control programs for a sustainable system of Plant diseases caused by *Xanthomonas* spp., The Inhibitory activity of *C. rohlfsianum* may be due to its content of alkaloids the presence of phenolics, triterpenoids, saponins, steroidal, compounds, Kaempferol, Genistein, Hesperetin, Oleanolic acid and 4, 7, 8-Trihydroxyflavone. (Elabbar *et al*., 2014). The results also showed the tuber extract was more efficient than the leaves extract, this result agreed with (Lee *et al*., 2016) when using the Tubers and leaves of a type of seaweed Zostera marina against some microbial species. The inhibitory activity increased with an increase in concentration, the concentration of 400mg/ml was the most effective against plant pathogenic bacteria. *X. campestris* pv. *vesicatoria* was

the most resistant, *X.axonopodis* pv. *eucalyptus* was the most sensitivity to aqueous extracts of *Cyclamen rohlfsianum*.

5 Conclusions

This study concludes that *Cyclamen rohlfsianum* (tubers and leaves) possesses inhibitory activity against the plant pathogenic bacteria, and the most efficacy is reached with increasing concentration. The best inhibition activity of aqueous extracts against tested bacteria was obtained with 400 mg/mL. Commonly, *X.campestris* pv. *vesicatoria* was the most resistant, while *X.axonopodis* pv. *eucalyptus* was the most sensitivity to aqueous extracts. Therefore, Data in this work indicated that the use of *Cyclamen rohlfsianum* could be a valid alternative for bio-control of plant pathogenic bacteria.

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Conflict of Interest: The authors declare that there are no conflicts of interest.

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