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Original Research Article

Efficacy of Antibacterial Chemicals and Essential Oils against *Xanthomonas oryzae* pv. *oryzae* under *In vitro* Conditions

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A B S T R A C T

Bacterial leaf blight (BLB) of rice caused by *Xanthomonas oryzae* pv. *oryzae* (Uyeda & Ishiyama) is emerging as serious threat to worldwide Rice production. The present study was conducted to investigate the efficacy of antibacterial chemicals and essential oils against *Xanthomonas oryzae* pv. *oryzae* by agar well method. The results revealed that among the antibiotics tested, Streptomycin showed highest antibacterial activity at all the three concentrations (250ppm, 500ppm and 1000ppm) produced inhibition zone 18.43mm, 21.43mm and 25.43mm respectively. While, Tricyclazole showed highest inhibition zone of 10.1mm,14.7mm and 17.7mm at 0.05%, 0.1% and 0.2% concentration. Among the six essential oils tested, lemon grass oil showed highest inhibitory activity at 2000ppm (16.76mm) followed by citronella oil at 500ppm (12.1mm) and (13.4mm) at 1000ppm.

Keywords

BLB, antibiotics, Chemicals and essential oils

Introduction

Bacterial leaf blight (BLB) of rice, caused by *Xanthomonas oryzae* pv. *oryzae* (Ishiyama) (Swings *et al.*, 1990), is the major limiting factor in rice production. The disease can damage the crop (Mew, 1987) on large scale and can cause 30 to 50 % yield loss (Reddy, 1989 and Adhikari *et al.*, 1994). It is the most devastating disease of rice in both irrigated and rainfed ecosystem.

Chemicals prevent rice diseases which can result in severe damage to the crop in terms of both quality and quantity. In India streptomycin mixture was tested for disinfection of rice seeds and was found effective (Srivastava, 1972). Bleaching powder containing 30% chlorine (2kg/ha) significantly reduced the BLB lesion in rice (Chand *et al.*, 1979). It has been observed that acetylenic compounds such as dicarbamoylacetylen (Collocidin) at low concentrations completely inhibits *Xanthomonas oryzae* in liquid medium (Okimoto and Misato, 1963). But, the chemicals has its toxic residual effects on plants, soil and ultimately on human health. The essential oils have gained popularity as these are found effective against bacterial and fungal pathogens. Therefore, the present study was aimed to find out the efficacy of

the antibacterial chemicals and essential oils against the pathogen under laboratory condition.

Materials and Methods

Evaluation of antibacterial chemicals against *Xanthomonas oryzae* pv. *oryzae*

Antibiotics viz, streptomycin, tetracycline, chloramphenicol and Plantomycin were assessed at 250, 500 and 1000ppm and chemicals viz Tricyclazole, azoxystrobin, and carbendazim+mancozeb were assessed at (0.05%, 0.1% and 0.2%concentrations) against *Xanthomonas oryzae* pv. *oryzae* by the agar well method. The sterilized petriplates is poured with nutrient agar medium and allowed to solidify. The bacterial suspension of *Xanthomonas oryzae* pv.*oryzae* was evenly spread over the media surface by means of sterilized spreader. Thereafter, 5 mm diameter well was made in each agar plate by using sterilized cork borer. The required concentration of antibacterial chemicals was loaded into the each well (50µl/well) in petri plates separately with the help of micropipette. The plate without any chemical is treated as control. The experiment was performed in triplicate under aseptic conditions. The petri plates were then kept in a BOD incubator at 28± 1^0C for 48hours. The inhibition zone was measured in mm with the help of a scale after 48 h of incubation.

Evaluation of essential oils against *Xanthomonas oryzae* pv. *oryzae*

Six essential oils viz., Neem oil (*Azadirachta indica*), lemongrass oil (*Cymbopogon flexuosus*), cedarwood oil (*Cedrus deodara*), eucalyptus oil (*Eucalyptus globulus*), Citronella oil (*Ciybopogan nardus*) and clove oil (*Syzgium aromaicum*) were tested for their bactericidal properties against *Xanthomonas oryzae* pv. *oryzae*. The essential oils were evaluated at three different concentrations of 500ppm, 1000ppm and 2000ppm. To prepare 0.3% emulsifier, 3ml of emulsifying agent was added to 997ml of sterilized water. 4000ppm stock solution of each essential oil was formulated by dissolving 0.5 ml of essential oil in 124.5ml of 0.3% emulsifying agent. From the stock solution, desired concentrations were prepared.

In this method nutrient agar medium is poured in an sterilized petri plates and enabled them to solidify. Afterwards, 100µl of 48h old bacterial suspension of *Xanthomonas oryzae* pv.*oryzae* was pipetted and placed at the centre of the solidified petriplate and the bacterial suspension was evenly spread over the media surface by means of sterilized spreader. 5 mm diameter well was made in each agar plate by using sterilized cork borer. The required concentration of essential oils was loaded into the each well (50µl/well) in petri plates separately with the help of micropipette. The control plate is prepared in which the well is loaded by sterile distilled water. The experiment was performed in triplicate under aseptic conditions. The petriplates were then shifted to the BOD incubator for an incubation at 28± 1^0C for 48hours. The inhibition zone was measured in mm with the help of a scale after 48 h of incubation. The experiment was conducted with three replications for each treatment and the data was analyzed in a completely randomized design.

Results and Discussion

Sensitivity of antibacterial chemicals and essential oils against the pathogen was conducted through agar well method.

Data pertaining to inhibition zone (mm) are depicted in Table 1. Among the antibiotics

tested, Streptomycin showed highest antibacterial activity at all the three concentrations (250ppm, 500ppm and 1000ppm) produced inhibition zone of 18.43mm, 21.43mm and 25.43mm, followed by Chloramphenicol with an inhibition zone of 15.1mm, 20.76mm and 22.43mm respectively. The least inhibition zone was recorded by Plantomycin. Zone of inhibition was not observed in the control plate.

The results of table 2 indicated that the Tricyclazole showed highest inhibition zone of 10.7mm, 14.7mm and 17.7mm at 0.05%, 0.1% and 0.2% concentration. The statistical analysis also showed that Tricyclazole was found to be best at all the three concentration that is 0.05%, 0.1% and 0.2% concentration. It was followed by carbendazim+mancozeb combination (10.1mm, 14.4mm and 16.7mm) at 0.05%, 0.1% and 0.2% respectively. Azoxystrobin showed the minimum inhibition zone of (10.1mm, 13.4mm and 14.7mm) at 0.05%, 0.1% and 0.2% respectively.

Similar findings were in accordance with Prasad *et al.,* (2018) who evaluated six antibiotics among which streptomycin recorded the highest inhibition zone of 27mm at 0.05% and 26.83mm at 0.03% concentration. Swati *et al.,* (2015) conducted experiment in vitro by using five chemicals and observed that the Tricyclazole 75 WP (Beam) recorded maximum inhibition zone.

Data presented in Table 3 revealed that the Lemon grass oil showed highest inhibitory activity at 2000ppm(16.76mm) followed by Eucalyptus oil of 15.4mm. At 1000ppm, citronella oil showed high inhibition of 13.4mm. The results showed that the citronella oil and clove oil gave the good result of (12.1mm) at 500ppm and 1000ppm (13.4mm).

Bibiana *et al.,* (2012) reported the efficacy of the lemongrass oil having good antibacterial effects contrary to the gram-negative bacteria. Wonni *et al.,* (2016)reported that among the three essential oils i.e., *C. citratus*, *E. camaldulensis* and *M. piperita* tested *in vitro* contrary to *Xanthomonas oryzae* pv. *oryzae*, *C. citrates* oil showed the highest inhibition zone (30 mm) at 1:5 dilution (v/v).

Table.1 Efficacy of antibiotics against *Xanthomonas oryzae* pv. *oryzae*

S.No.	Antibiotics	Inhibition zone in mm			Mean
		250ppm	500ppm	1000ppm	
1.	**Chloramphenicol**	15.1	20.7	22.4	19.4
2.	**Streptomycin**	18.4	21.4	25.4	21.7
3.	**Tetracycline**	10	13.4	16.7	13.4
4.	**Plantomycin**	9.4	11.4	13.4	11.4
5	**Control**	0	0	0	-
Mean		10.6	13.4	15.6	-

Factors	CD at 5%	SEm±
Antibiotic(A)	0.96	0.33
Concentration(B)	1.28	0.43
Interaction(A×B)	2.16	0.74

Table.2 Efficacy of Chemicals against *Xanthomonas oryzae* pv. *oryzae*

S.NO	Chemicals	Inhibition zone in (mm)			Mean
		0.05%	0.1%	0.2%	
1	Tricyclazole	10.7	14.7	17.7	14.4
2	Azoxystrobin	10.1	13.4	14.7	12.7
3	Carbendazim+ Mancozeb	10.1	14.4	16.7	13.7
4	Control	0	0	0	-
Mean		7.7	10.6	12.3	-
Factors		CD at 5%		SEm±	
Chemicals(A)		0.61		0.21	
Concentration(B)		0.71		0.24	
Interaction(A×B)		1.23		0.4	

Table.3 *In vitro* evaluation of essential oils against *Xanthomonas oryzae* pv. *oryzae*

S.No.	Essential oil	Inhibition zone in (mm)			Mean
		500ppm	1000ppm	2000ppm	
1	Neem oil	11.7	12.7	15.1	13.2
2	Lemon grass	10.4	13.1	16.7	13.4
3	Cedar wood	11.1	10.4	11.1	10.8
4	Eucalyptus	11.7	12.7	15.4	13.3
5	Citronella	12.1	13.4	14.7	13.4
6	Clove oil	12.1	13.1	14.7	13.3
7	Control	0	0	0	-
Mean		9.8	10.8	12.5	-
Factors		CD at 5%		SEm±	
Essential oil(A)		0.42		0.14	
Concentration(B)		0.64		0.22	
Interaction(A×B)		1.12		0.39	

Fig.1 Inhibition zone produced by antibiotics against *Xanthomona soryzae* pv.*oryzae*

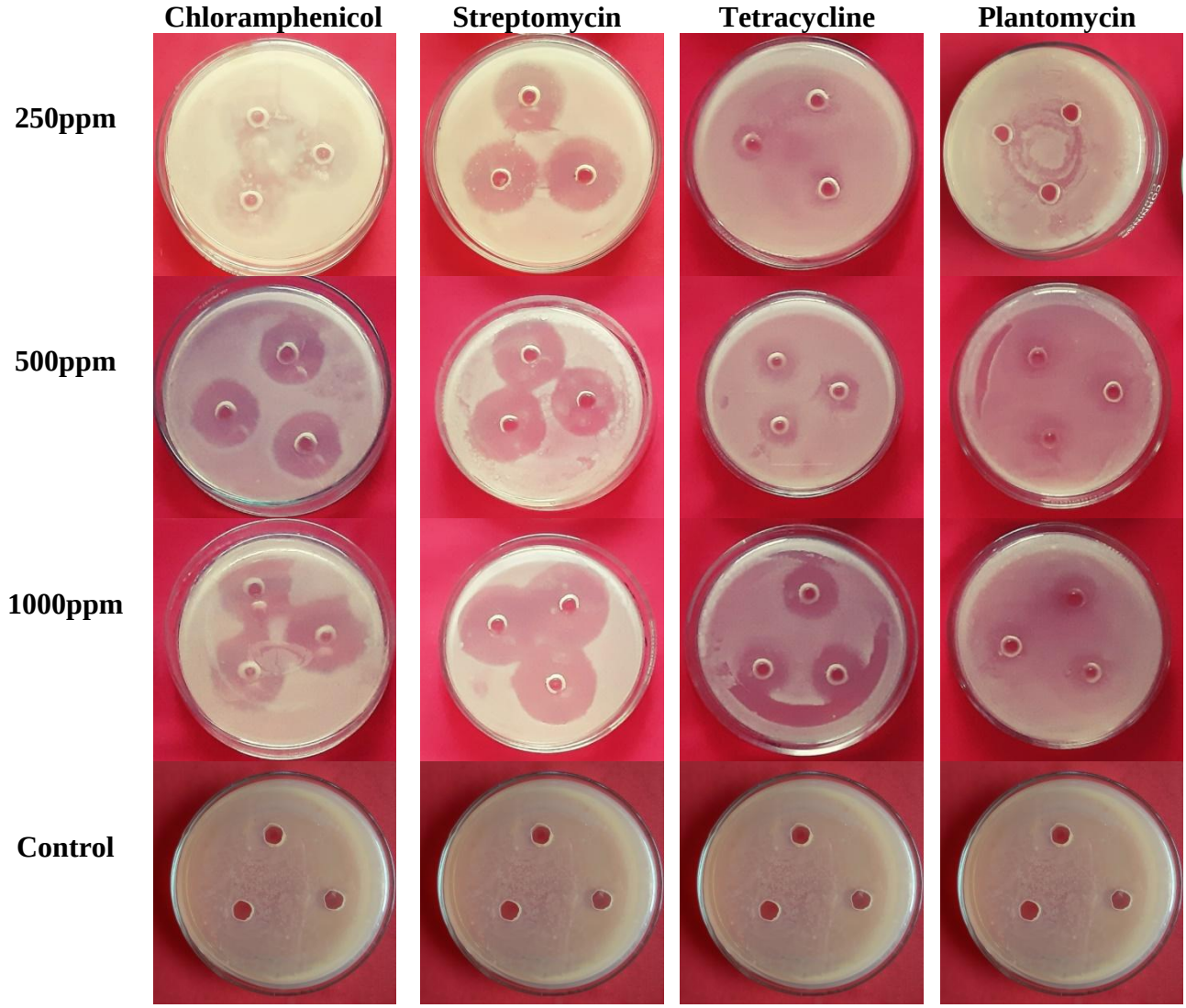


Fig.2 Inhibition zone produced by chemicals against *Xanthomonasoryzae*pv.*oryzae*.

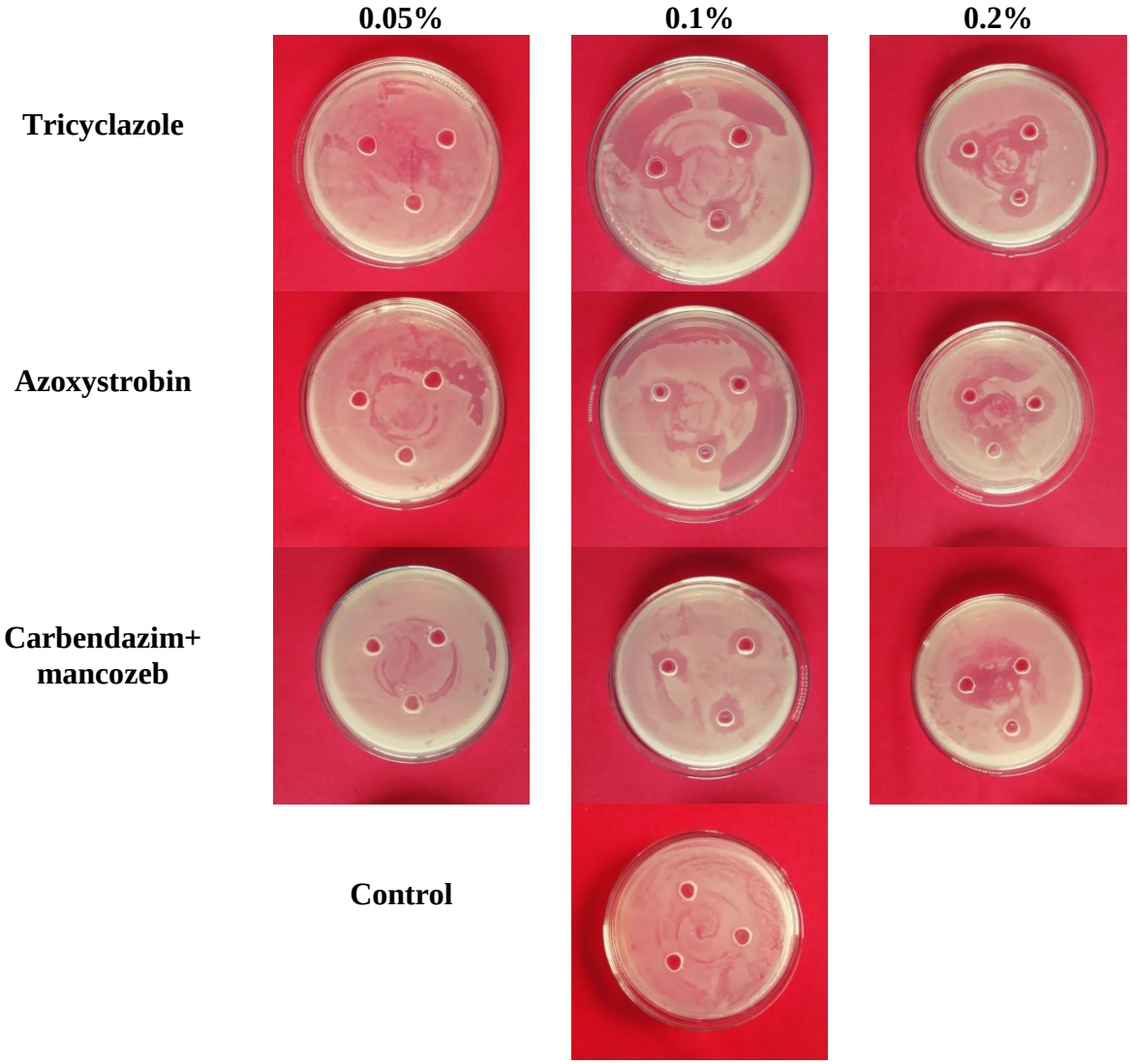
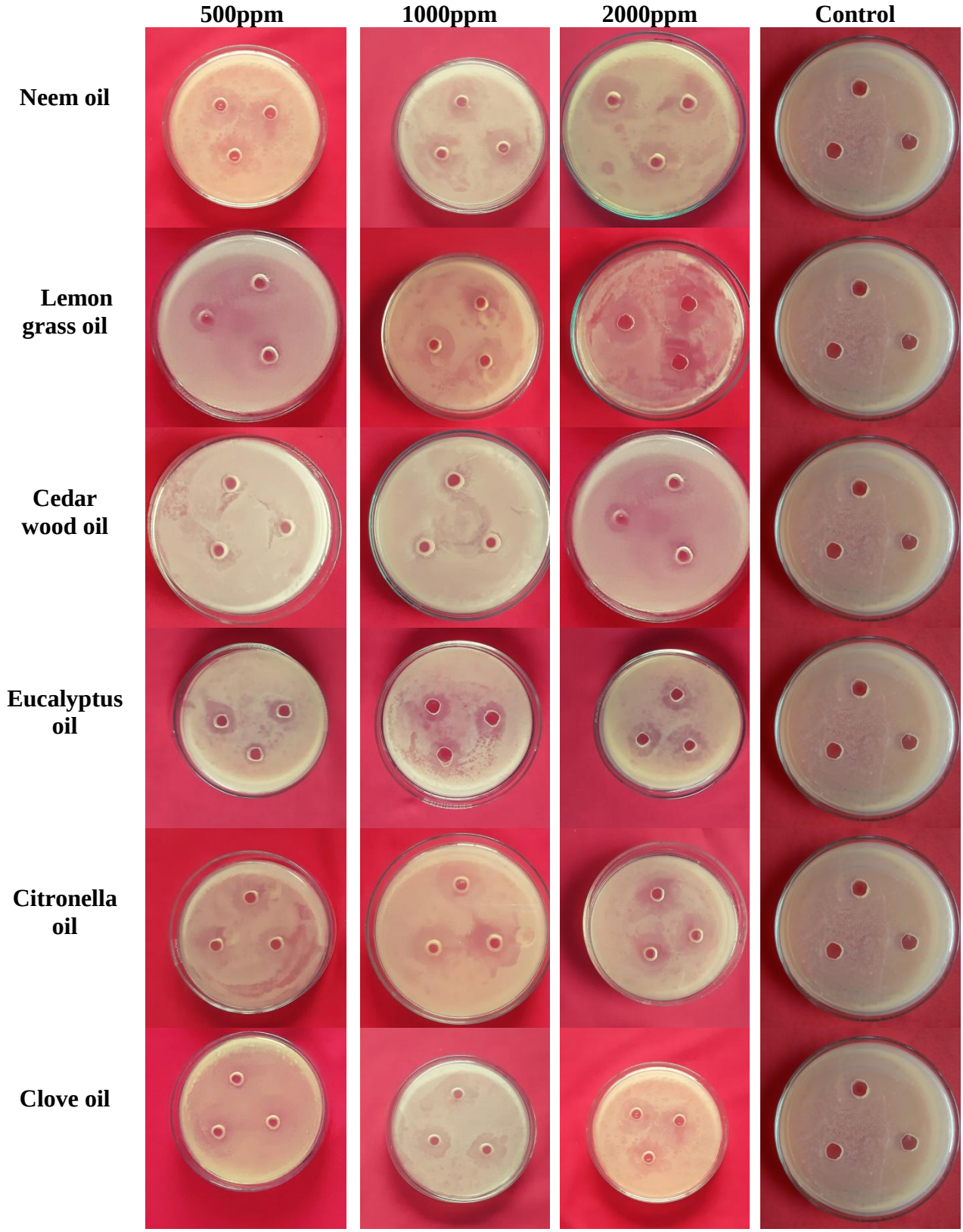


Fig.3 Inhibition zone produced by essential oils against *Xanthomonasoryzae*pv.*oryzae*.



Seven antibacterial compounds (3 antibiotics & 3 chemicals) and six essential oils were evaluated against *Xanthomonas oryzae* pv.*oryzae*. Considering the results it can be said that among the antibiotics Streptomycin showed highest antibacterial activity at all the three concentrations (250ppm, 500ppm and 1000ppm) produced inhibition zone 18.43mm, 21.43mm and 25.43mm respectively. Among the three chemicals, Tricyclazole showed highest inhibition zone of 10.7mm, 14.7mm and 17.7mm at 0.05%, 0.1% and 0.2% concentration. Among the essential oils tested, Lemon grass oil showed highest inhibitory activity at 2000ppm (16.76mm). Citronella oil gave the good result at 500ppm (12.1mm) and 1000ppm (13.4mm).

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