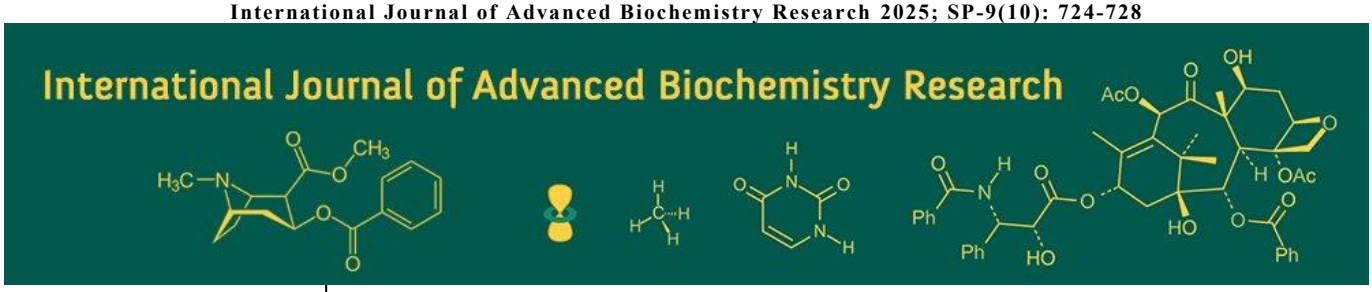

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In vitro evaluation of antibiotics against *Enterobacter cloacae* causing leaf rot of onion

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Abstract
A present investigation for the management of leaf rot of onion (*Allium cepa*) was carried out at Department of Plant Pathology, University of Horticultural Sciences Bagalkot. The antibiotics / chemicals were screened against the growth of *Enterobacter cloacae,* by agar well diffusion method. Among the different antibiotics, streptocycline (31.33, 33.50 and 35.33 mm) and plantamycin (26.00, 26.83 and 28.33 mm) were shown highest inhibition at 300, 400 and 500 ppm concentrations, respectively. It was followed by 2-bromo-2 nitropropane-1, 3-diol (23.17, 25.83 and 31.67 mm) at different concentrations, respectively. Among the fungicides copper hydroxide (16.17, 18.83 and 22.17 mm) and copper oxy chloride (15.83, 17.50 and 20.83 mm) recorded highest inhibition at 1500, 2000 and 2500ppm, respectively. Whereas kasugamycin (Omycin) was not shown any inhibition zone at all the concentrations tested. Wheras in water control no inhibition was observed.

Keywords: *In-vitro, Enterobacter cloacae,* inhibition zone, agar well diffusion method, antibiotics

Introduction
Onion (*Allium cepa* L.) is one of the oldest bulb crops belongs to Amaryllidaceae family. The genus Allium comprises over 700 species which can be found throughout the tropical, temperate and sub-temperate regions of the world (Fritsch and Friesen, 2002) [2]. There are five important species of Allium of which the onion (*Allium cepa*) is the major cultivated species grown all over the world. According to Vavilov (1951) [7] the primary center of origin lies in central Asia. Onion has manifold use such as spice, vegetable, salad-dressing etc., (Vohra *et al*., 1974) [4]. Among vegetables, onion often called as "queen of kitchen" it is one of the oldest known and an important crop. India (288.00 lakh tons) is the largest producer of onion. In India Maharashtra is the leading producing state. In Karnataka the area under onion is 2.32 lakh hectares, with production of 38.91 lakh tons, (Anon., 2024) [1]. Onion has high value both in domestic use and export purpose, but growing of onion has some constraints such as insect pests and diseases. The pests are thrips, whiteflies, beetles etc. Now a day's bacterial leaf rot is becoming an important disease in northern part of Karnataka from past 8-10 years. There is a need to develop ecofriendly management practice for mitigating the plant diseases. Hence, commercially available bio-agents and botanicals were evaluated against the disease under *in vitro*.

Materials and Methods
Evaluation of different Antibiotics
Antibiotics were screened under *in vitro* against the growth of bacterium causing leaf rot of onion at different concentrations by agar well diffusion method. The list of chemicals which were evaluated with three replications in each treatment is mentioned in Table 1.
The bacterium was multiplied by inoculating the fresh culture into 50 ml of nutrient broth taken in a 100 ml Elsmere's flask. The flask which is inoculated with the pathogen was kept in a shaker incubator at 30^0C for 48 hours. The suspension of the bacterial culture was then poured into to the lukewarm nutrient agar medium (1000 ml) *i.e.* 5ml / litre in a conical flask. The medium was seeded with bacteria was poured into the sterilized Petri plates and plates were allowed to solidify.
The chemicals and antibiotics were prepared at different concentrations as mentioned in the list.

The Agar well diffusions measuring 10 mm in diameter were made after solidification of the seeded medium in the Petri plates and then poured with the respective chemical solution into the wells. The inoculated plates were incubated at 28 ± 2°C for 24h and observed for the production of inhibition zone around agar well diffusions. The results obtained by the experiment were analyzed statistically.

Results

The antibiotics / chemicals were screened against the growth of *Enterobacter cloacae,* by agar well diffusion method. Among the different antibiotics, streptocycline (31.33, 33.50 and 35.33 mm) and plantamycin (26.00, 26.83 and 28.33 mm) were shown highest inhibition at 300, 400 and 500 ppm concentrations, respectively. It was followed by 2-bromo-2 nitropropane-1, 3-diol (23.17, 25.83 and 31.67 mm) at different concentrations, respectively. Among the fungicides copper hydroxide (16.17, 18.83 and 22.17 mm) and copper oxy chloride (15.83, 17.50 and 20.83 mm) recorded highest inhibition at 1500, 2000 and 2500ppm, respectively. Whereas kasugamycin (Omycin) was not shown any inhibition zone at all the concentrations tested. Wheras in water control no inhibition was observed (Table 2 and Plate 1).

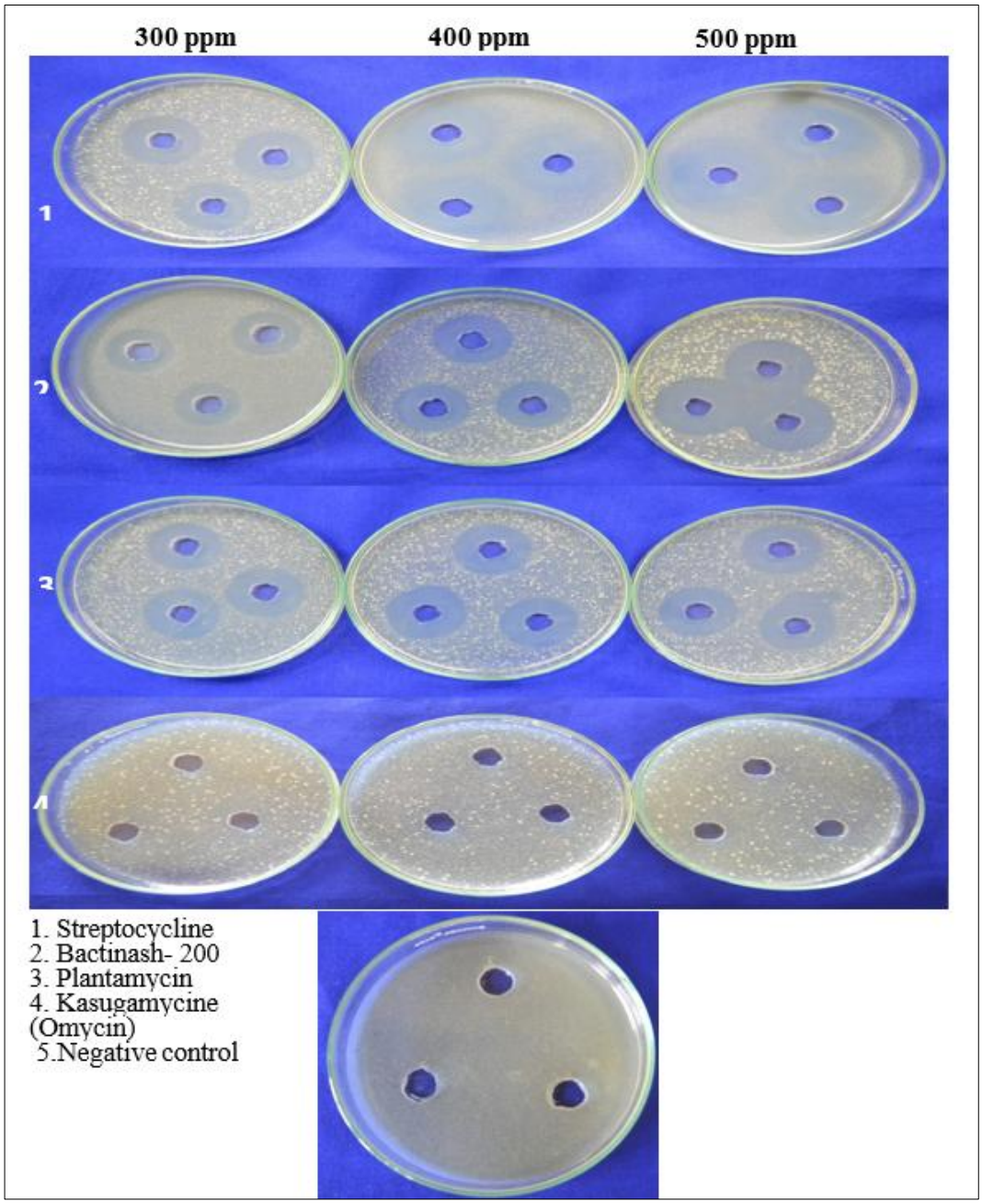

Plate 1: *In vitro* screening of antibiotics against *Enterobacter cloacae*

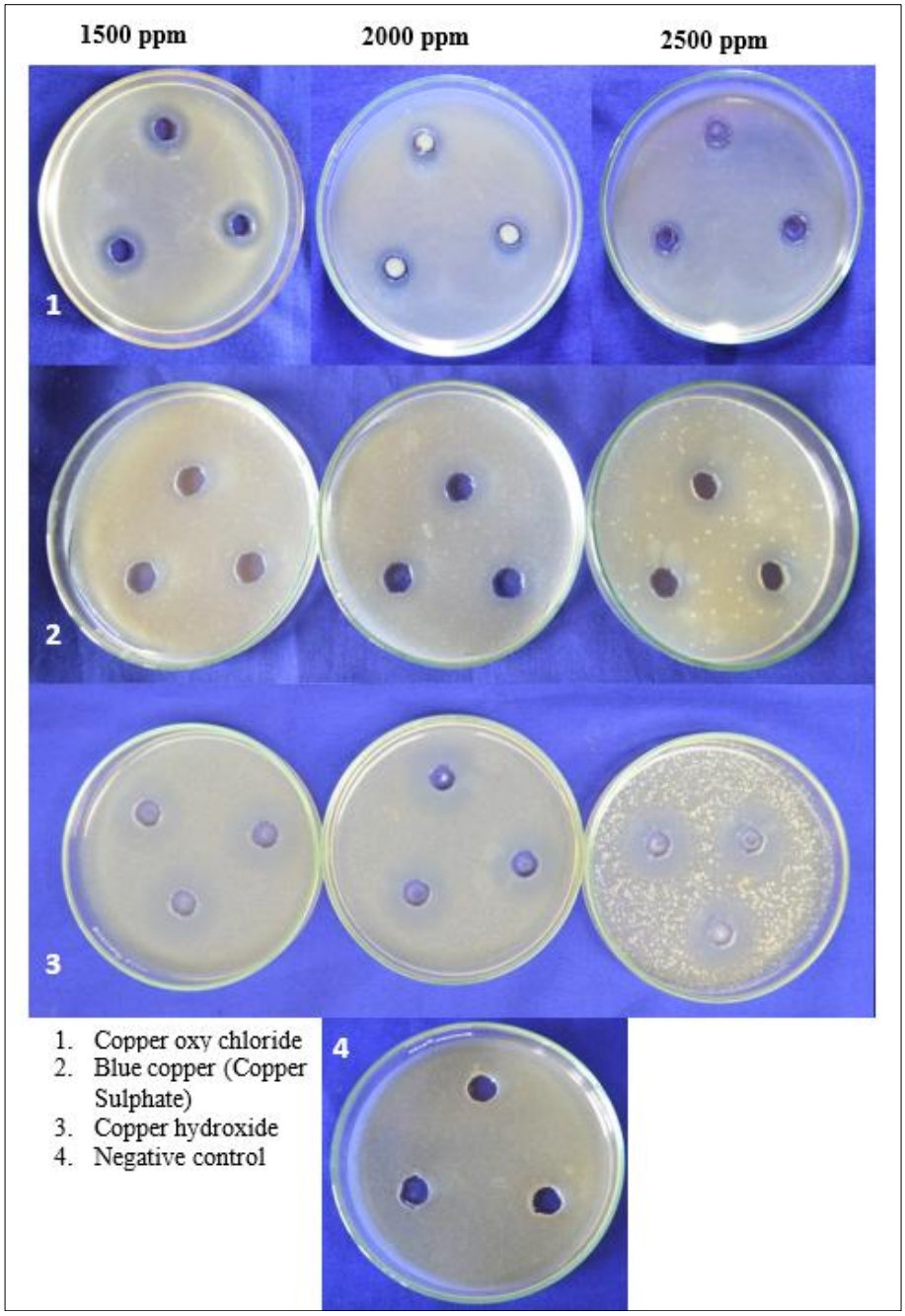

Plate 2: *In vitro* screening of fungicides against *Enterobacter cloacae*

Table 1: List of chemicals used for *in-vitro* evaluation against *Enterobacter cloacae* of onion

Sl. No.	Chemicals/ Antibiotics used	Chemical composition	Name of the manufacturer	Concentrations(ppm)		
1	Bactinash - 200	2-Bromo - 2 nitropropane-1,3-diol 95% w/w	Multiplex Agricare Pvt. Ltd. Hyderabad, Telangana.	300	400	500
2	Plantomycin®	(Streptomycin sulphate 9% + Tetracycline hydrochloride 1%)	Aries agro limited, Mumbai, Maharashtra	300	400	500
3	Omycin™	Kasugamycin-G- 3% S. L	M/S Biostadt India, Mumbai, Maharashtra	300	400	500
4	K-cycline	(Streptomycin Sulphate + Tetracycline Hydrochloride) 9:1 SP	Karnataka agro chemicals, Banglore, Karnataka	300	400	500
5	Kocide® 101	Copper hydroxide 77% W. P	E.I. Dupont de Nemours & Company, michigan, United state	1500	2000	2500
6	Blitox®	Copper oxy chloride 50% W. P	Tatarallies enterprise, Wadala, Mumbai	1500	2000	2500
7	Copper sulphate	Copper sulphate	-	1500	2000	2500

Table 2: Effect of antibiotics and copper fungicides against *Enterobacter cloacae* under *in vitro* condition, Effect of antiiotics against *Enterobacter cloacae* under *in vitro* condition

Sl. No.	Antibiotics	Mean zone of inhibition (mm)			
		300 ppm	400 ppm	500 ppm	Mean
1	Bactinash-200	23.17 (4.92)	25.83 (5.18)	31.67 (5.72)	26.89 (5.27)
2	Plantamycin	26.00 (5.20)	26.83 (5.28)	28.33 (5.42)	27.06 (5.30)
3	Streptocycline (K-Cyclin)	31.33 (5.69)	33.50 (5.87)	35.33 (6.03)	33.39 (5.86)
4	Kaugamycin-G	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
5	Control	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
	Mean	16.10 (3.56)	17.23 (3.67)	19.07 (3.83)	
		Antibiotics	Concentration	Antibiotics X Concentration	
	SEm±	0.14	0.11	0.25	
	CD @ 1%	0.42	0.32	0.73	

*Figures in the parenthesis are square root transformed

Table 3: Effect of copper fungicides against *Enterobacter cloacae* under *in vitro* condition

Sl. No.	Copper fungicides	Mean zone of inhibition (mm)			
		1500 ppm	2000 ppm	2500 ppm	Mean
1	Copper sulphate	12.67 (3.70)	15.17 (4.02)	19.00 (4.47)	15.61 (4.06)
2	Copper hydroxide	16.17 (4.14)	18.83 (4.45)	22.17 (4.81)	19.06 (4.47)
3	Copper oxy chloride	15.83 (4.10)	17.50 (4.30)	20.83 (4.67)	18.06 (4.36)
4	Control	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00)
	Mean	11.17 (3.24)	12.88 (3.44)	15.50 (3.74)	
		Antibiotics	Concentrations	Antibiotics X Concentrations	
	SEm±	0.21	0.53	0.19	
	CD @ 1%	0.61	1.05	0.37	

*Figures in the parenthesis are square root transformed

Discussion

In vitro evaluation of chemicals or antibiotics against the growth of *Enterobacter cloacae*

A total of seven chemicals were evaluated for their efficacy against growth of *Enterobacter cloacae* by agar well diffusion method and the mean inhibition diameter was recorded.

Among the different antibiotics, streptocycline (33.39 mm) and plantamycin (27.06 mm) were shown the highest average inhibition at 300, 400 and 500 ppm concentrations respectively. This was followed by 2-bromo-2 nitropropane-1, 3-diol (26.89 mm respectively). Among the fungicides, copper hydroxide (19.06 mm) and copper oxy chloride (18.05 mm) recorded highest inhibition at 1500, 2000 and 2500 ppm respectively. However, kasugamycin showed no inhibition zone in all the concentrations tested (Figure 3).

Similar kinds of investigations were also reported on banana by Thammaiah *et al.* (2005) [5] through *in vitro* screening of chemicals against *Erwinia chrysanthemi* by the modern paper disc method with seven treatments. The result showed that the combination of streprocycline at 1000 ppm + copper oxy chloride at 2000 ppm, recorded the maximum inhibition (24.00 mm) followed by copper oxy chloride at 4000 ppm (23.33 mm).

Nagrale *et al.,* (2013) [6] studied the effectiveness of different chemicals against *E. chrysanthemi pv. paradisiaca* under *in vitro* conditions. The maximum inhibition zone was noticed for streptocycline (200 ppm) + copper oxychloride (0.1%), tetracycline (200 ppm) and streptocyline (200 ppm) *viz.,* which is followed by agrimycin (200 ppm), bordeaux mixture (0.1%) gentamycin (200 ppm), captan (0.2%) and copper oxychloride (0.25%).

Conclusion

Among the different antibiotics evaluated against the *E. cloacae*, streptocycline (33.39 mm) shown highest mean zone of inhibition. Among the fungicides, copper hydroxide (19.06 mm) recorded highest mean zone of inhibition.

The results on evaluation of chemicals, botanicals and bio agents against leaf rot disease revealed that, among the treatments, T_1 (Streptocycline at 500 ppm + Copper oxy chloride (0.3%) was found most effective. In which the percent reduction of disease in terms of percent disease incidence (77.83%) and number of lesions per branch (76.24%).

Acknowledgments

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References

1. Anonymous. NHB data base. 2024.
2. Fritsch KM, Friesen N. Evolution, domestication and taxonomy. In: Rabinowitch HD, Currah L, editors. *Allium* crop science, recent advances. Oxon: CAB International; 2002. p. 5-30.
3. Priya RS, Geetha D, Ramesh PS. Antioxidant activity of chemically synthesized AgNPs and biosynthesized *Pongamia pinnata* leaf extract mediated AgNPs - a comparative study. Ecotoxicol Environ Saf. 2015;134:308-18.
4. Vohora SB, Rizman M, Khan JA. Medicinal uses of common Indian vegetables. Planta Medica. 1974;23:381-93.
5. Thammaiah N, Kanamadi VC, Shirol AM, Satyanarayana Reddy B. Management of rhizome rot or tip over disease of banana caused by *Erwinia chrysanthemi*. J Asian Hort. 2005;2(1):62-3.
6. Nagrale DT, Suresh NG, Shailesh P, Gawande AKM, Satish AR. Characterization of a bacterial collar and rhizome rot of banana (*Musa paradisiaca*) caused by

strains of *Erwinia chrysanthemi* pv. *paradisiaca*. J Appl Nat Sci. 2013;5(2):435-41.
7. Vavilov NI. The origin, variation, immunity and breeding of cultivated plants. Chronica Botanica. Waltham (MA): USA; 1951.
8. Vohora SB, Rizman M, Khan JA. Medicinal uses of common Indian vegetables. Planta Medica. 1974;23:381-93.