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In-vitro evaluation of chemicals/antibiotics against the growth of Tip over disease of banana (*Erwinia chrysanthemi*)

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Abstract
Among the chemicals tested, copper oxychloride at 4000 ppm was found to be effective in inhibiting the growth of the pathogen (23.16mm) followed by 3000 ppm of copper oxychloride (21.33mm) and 2000 ppm of copper oxychloride (17.33mm). Among the antibiotics tested, streptocycline1000 ppm was found to be effective in inhibiting the growth of the pathogen (21.50mm) followed by 750 ppm of streptocycline produced with an inhibition zone of 20.16mm and 500 ppm of streptocycline produced with an inhibition zone of 19.33mm. Combination of antibiotics and chemicals tested, streptocycline + copper oxychloride produced the highest inhibition zone of 24.75mm at 500 + 3000 ppm followed by streptocycline + copper oxychloride produced an inhibition zone of 19.75mm at 500+1000 ppm and streptocycline +copper oxychloride produced an inhibition zone of 19.37mm at 500+2000 ppm.

Keywords: *In-vitro*, chemicals/antibiotics, *Erwinia chrysanthemi*

Introduction
Banana (*Musa* sp) is one of the important fruit crops of the world as well as India. Banana called as "Adam's fig" and "Apple of Paradise" belonging to the family Musaceae and the genus *Musa*. Bananas are widely grown in India with great socio-economic significance, interwoven in the cultural heritage of the country. It is known to be one of the earliest fruit crops grown by mankind at the dawn of civilization considering its nutritive value. (Radha and Mathew, 2007) [7]. Wardlaw (1950) [11] for the first time reported the bacterial nature of bacterial head rot or rhizome rot of banana from Honduras. Edward *et al.* (1973) [2] reported the tip-over disease of banana from Allahabad in Utter Pradesh. Dickey and Victoria (1980) [1] reported that the disease caused 80-90 per cent reduction of production within five years after initial infection in a plantation. Lakshmanan and Mohan (1986) [3] recorded nearly 40-60 per cent loss in three to five month old crops. Later in 1992, they reported that the incidence ranged from 25-45 per cent and in extreme cases it was high as 60-80 per cent, in some fields. Nagaraj *et al*. (2012) [6] reported that the disease incidence ranged from 30-35 per cent in the districts of Bangalore and Kolar of Karnataka state. Hence, present studies were under taken to evaluate the efficacy of chemicals/antibiotics and combination of chemicals and antibiotics against the growth of the *Erwinia chrysanthemi*.

Material and Methods
An *in-vitro* experiment was conducted during 2012 at K. R. C. College of Horticulture, Arabhavi to find out suitable chemicals/antibiotics against growth of *Erwinia chrysanthemi.*
A heavy suspension of *Erwinia chrysanthemi* (Muddebihal isolate) ($7x10^8$cfu/ml) was seeded to the sterilized nutrient agar medium by mixing the bacterial culture with the molten nutrient agar (50^0C) in a 500ml Erlenmeyer flask. The seeded medium was poured on to sterilized petriplates and allowed to solidify. Previously sterilized filter paper discs (Whatman No.1) measuring 6 mm diameters were soaked in different chemical/antibiotic solution for 10 minutes in sterilized petriplates. The excess solution from the filter paper disc was removed by touching side of the paper discs to the wall of the beaker containing solution. Then the filter paper discs were placed on the surface of seeded nutrient agar medium contained in the petriplates. The inoculated plates were kept in the refrigerator at 4^0C for four hours so as to allow the diffusion of the chemicals in the medium and then plates were taken out and

inoculated at 28^0C for 48 hours. Zone of inhibition produced around the filter paper discs was recorded. Filter paper discs dipped in sterile water served as check.

Result and Discussion

The results are presented in the Table 1, Table 2, Plate 1 and Plate 2 revealed that copper oxychloride at 4000 ppm was found to be effective in inhibiting the growth of the pathogen with an inhibition zone of 23.16mm followed by 3000 ppm of copper oxychloride 21.33mm and 2000 ppm of copper oxychloride 17.33mm. However, copper sulphate at 2000 ppm was moderately effective in inhibiting the growth of the pathogen with an inhibition zone of 16.50mm followed by 1000 ppm copper sulphate produced with an inhibition zone of 15.66mm and 500 ppm copper sulphate produced with an inhibition zone of 13.50mm. The treatment bleaching powder was not effective at 1000 ppm and 2000 ppm concentration but at higher concentration 3000 ppm it produced an inhibition zone of 6.00mm. The treatment sodium hypochlorite produced slightly inhibition zone at 500 ppm (6.66mm) followed by 100 ppm sodium hypochlorite produced with an inhibition zone of 4.33mm and 250 ppm produced with an inhibition zone of 3.66mm against the growth of the pathogen. These findings are in agreement with the reports of Thammaiah *et al.* (2006) [10] who reported that the combination of streptocycline 1000 ppm+ copper oxychloride 2000 ppm recorded the maximum inhibition of *Erwinia chrysanthemi* (24.00mm) followed by copper oxychloride 4000 ppm (23.33mm) under *in- vitro* condition. Saini and Parahar (1981) [8] have reported that stable bleaching powder at 1000 ppm was inhibitory to *Erwinia carotovora* sub sp. *carotovora.*

Among the antibiotics tested, streptocycline1000 ppm was found to be effective in inhibiting the growth of the pathogen (21.50mm) followed by 750 ppm of streptocycline produced with an inhibition zone of 20.16mm and 500 ppm of streptocycline produced with an inhibition zone of 19.33mm. While plantomycin produced an inhibition zone of 14.33mm, 13.00mm and 11.66mm at 1000 ppm, 750 ppm and 500 ppm concentrations respectively. Similar results were reported by Thammaiah *et al*. (2005) [9] who reported that Streptocycline 500 ppm and copper oxychloride 2000 ppm recorded maximum inhibition of the growth of the pathogen. Nagaraj *et al*. (2002) [5] reported that methoxy ethyl mercuric chloride at 2000ppm, copper sulphate at 4000ppm, streptocycline at 750ppm and norfloxin at 750ppm were found effective in inhibiting the growth of pathogen. The maximum inhibition was seen at 500 ppm of streptomycin sulphate against *Erwinia carotovora* subsp. *carotovora.* (Manoranjitham *et al*., 2010) [4].

The combination of antibiotics and chemicals tested, streptocycline + copper oxychloride produced the highest inhibition zone of 24.75mm at 500 + 3000 ppm concentration followed by streptocycline + copper oxychloride produced an inhibition zone of 19.75mm at 500+1000 ppm concentrations and streptocycline +copper oxychloride produced an inhibition zone of 19.37mm at 500+2000 ppm concentrations. While plantomycin + copper oxychloride produced an inhibition zone of 18.62mm at 500+1000 ppm concentrations. Similar results were recorded by Thammaiah *et al.* (2005) [9] who reported that streptocycline 500 ppm and copper oxychloride 2000 ppm recorded the maximum inhibition of *Erwinia chrysanthemi*. Combination of Streptocycline 1000 ppm + copper oxychloride 2000 ppm exhibited maximum inhibition (24.00mm) (Thammaiah *et al.*, 2006) [10].

Table 1: *In-vitro* evaluation of chemicals against *Erwinia chrysanthemi*

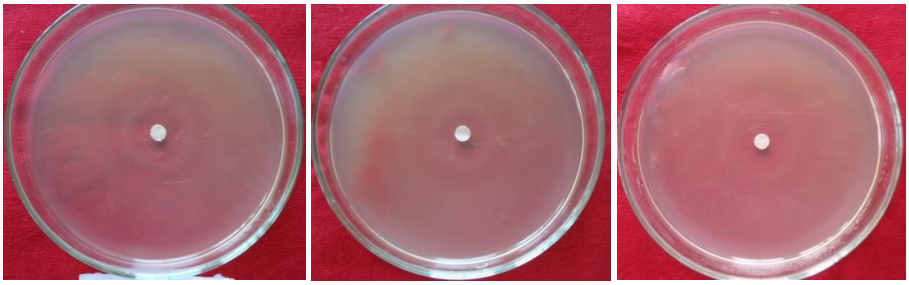
Treatments	Concentration (ppm)	Mean zone of inhibition (mm)
T_1- Streptocycline	500	19.33 (4.44)
	750	20.16 (4.53)
	1000	21.50 (4.68)
T_2- Copper oxychloride	2000	17.33 (4.19)
	3000	21.33 (4.65)
	4000	23.16 (4.85)
T_3- Copper sulphate	500	13.50 (3.72)
	1000	15.66 (4.01)
	2000	16.50 (4.12)
T_4- Plantomycin	500	11.66 (3.48)
	750	13.00 (3.67)
	1000	14.33 (3.84)
T_5- Bleaching powder	1000	0.00 (0.70)
	2000	0.00 (0.70)
	3000	6.00 (2.53)
T_6- Sodium hypochlorite	100	4.33 (1.99)
	250	3.66 (1.86)
	500	6.66 (2.66)
T_7- Control		0.00 (0.70)
S. Em±		0.23
CD at 1%		0.85

Figures in the parenthesis are the square root transformation values.

Table 2: *In-vitro* evaluation of antibiotics and chemicals against *Erwinia chrysanthemi*

Treatments	Concentration (ppm)	Mean zone of inhibition (mm)
T_1- Streptocycline+Copper oxychloride	500+1000	19.75 (4.50)
T_2- Streptocycline+Copper oxychloride	500+2000	19.37 (4.50)
T_3- Streptocycline+Copper oxychloride	500+3000	24.75 (5.02)
T_4-Plantomycin+Copper oxychloride	500+1000	18.62 (4.37)
T_5- Control		0.00 (0.70)
S. Em±		0.17
CD at 1%		0.46

Figures in the parenthesis are the square root transformation values.

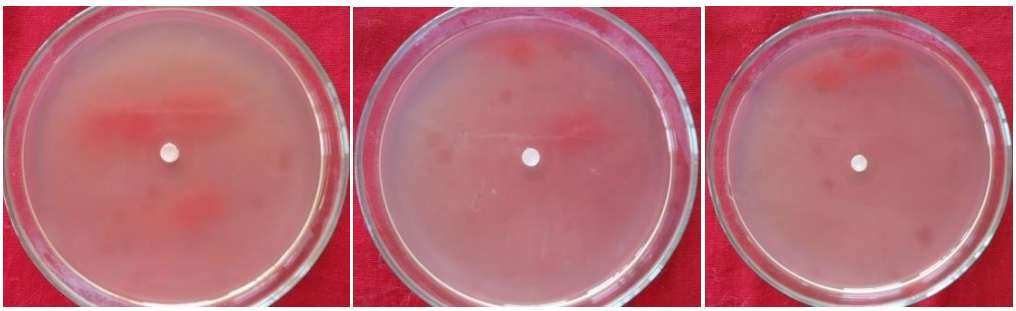


500ppm 750ppm 1000ppm

T_1-streptocycline

2000ppm 3000ppm 4000ppm

T_2-copper oxychloride



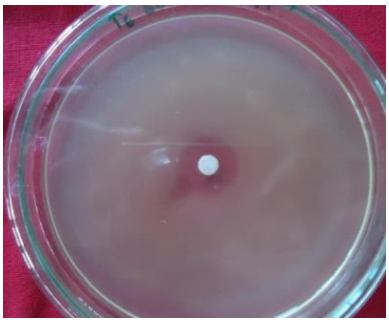
500ppm 1000ppm 2000ppm

T_3-copper sulphate

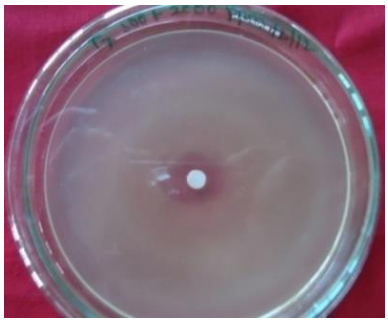
500ppm 750ppm 1000ppm

T_4-plantomycin

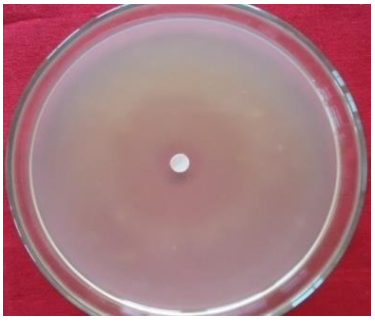
Plate 1: *In-vitro* evaluation of chemicals against *Erwinia chrysanthemi*



T_1-streptocycline 500ppm+ copper oxychloride 1000ppm



T_2- streptocycline 500ppm+ copper oxychloride 2000ppm



T_3- streptocycline 500ppm+ copper oxychloride 3000ppm

T_4- plantomycin 500ppm+ copper oxychloride 1000ppm

Control

Plate 2: *In vitro* evaluation of combination of chemicals and antibiotics against *Erwinia chrysanthemi*

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