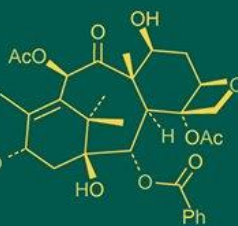
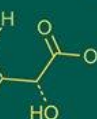
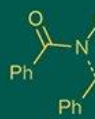


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In vitro efficacy of chemicals and antibiotics against *Pectobacterium* sp. causing soft rot disease in dragon fruit

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Abstract

Soft rot is an important postharvest and field-associated bacterial disease affecting the quality and marketability of dragon fruit. The present investigation was conducted to evaluate the *in-vitro* efficacy of selected chemicals and antibiotics against *Pectobacterium* sp., the bacterial pathogen associated with soft rot disease of dragon fruit. The antibacterial activity of eight chemicals at 1 and 2 percent concentrations and eight antibiotic treatments at 250 and 500 ppm was evaluated using the paper disc method. All the chemicals tested exhibited inhibitory activity against the pathogen, although the degree of inhibition varied considerably among treatments. At 1 percent concentration, copper sulphate recorded the maximum inhibition zone of 14.85 mm, followed by copper oxychloride (13.20 mm) and copper hydroxide (12.40 mm). At 2 percent concentration, copper hydroxide was the most effective treatment, producing an inhibition zone of 17.42 mm, followed by copper oxychloride (14.96 mm), ammonium bicarbonate (14.68 mm) and sodium hypochlorite (14.62 mm). Among the antibiotics, ciprofloxacin recorded the maximum inhibition zone of 16.66 mm at 250 ppm, whereas streptomycin + copper oxychloride was most effective at 500 ppm with an inhibition zone of 22.90 mm. Streptomycin alone recorded 20.36 mm inhibition at 500 ppm. Most treatments showed increased antibacterial activity with increasing concentration. The findings indicate that copper-based chemicals, particularly copper hydroxide and copper oxychloride, and antibiotic treatments containing streptomycin have considerable potential for suppressing *Pectobacterium* sp. under *in-vitro* conditions.

Keywords: Dragon fruit, soft rot, *Pectobacterium* sp., antibiotics, copper compounds, antibacterial activity, inhibition zone

1. Introduction

Dragon fruit [*Selenicereus monacanthus* (Lem.) D. R. Hunt], earlier known as *Hylocereus polyrhizus* (F. A. C. Weber) Britton & Rose, is a perennial, semi-epiphytic cactus belonging to the family Cactaceae (Patwary *et al.*, 2013) [8]. It originated in South and Central America and is now cultivated in many tropical and subtropical countries, including several parts of Asia (Barthlott and Hunt, 1993; Mizrahi and Nerd, 1999) [2, 6]. The recent taxonomic classification places *H. polyrhizus* under *S. monacanthus* (Govaerts *et al.*, 2021) [3]. Dragon fruit has gained importance due to its attractive fruit colour, nutritional value and good market demand. Among the different types, red-fleshed dragon fruit is widely preferred for commercial cultivation (Nangare *et al.*, 2020) [5]. The crop is generally propagated through stem cuttings for maintaining the desirable characters of the selected plant (Ortiz-Hernández, 1999) [7]. With the increase in area under dragon fruit cultivation, problems related to diseases are also becoming important. Bacterial soft rot is one such problem, particularly because infected fruits undergo rapid softening and decay, resulting in loss of fruit quality and market value. *Pectobacterium* spp. are important bacterial pathogens associated with soft rot and cause tissue maceration through the production of pectinolytic enzymes. The disease development is favoured by high moisture, wounds and suitable temperature conditions.

Management of bacterial soft rot is difficult once the infection becomes established, and therefore identification of effective chemicals and antibiotics is important.

Previous studies have reported good antibacterial activity of copper-based compounds such as copper hydroxide and copper oxychloride against *Pectobacterium* (Thiyagarajan, 2016; Pavitra *et al.*, 2025) [13, 9]. Streptomycin and other antibiotics have also been reported to inhibit *Pectobacterium* under laboratory conditions (Sang *et al.*, 2015; Shen *et al.*, 2025) [10, 11]. However, the effectiveness of these treatments may vary with the pathogen, concentration and environmental conditions. Hence, the present study was carried out to evaluate the *in-vitro* efficacy of selected chemicals and antibiotics against *Pectobacterium* sp. causing soft rot disease of dragon fruit.

2. Materials and Methods

2.1 Collection, isolation and maintenance of the pathogen

Diseased dragon fruit samples showing typical soft rot symptoms were collected from affected fruits and stems from infected plants. The collected samples were brought to the laboratory in clean polythene bags and processed for isolation of the associated bacterial pathogen. Small pieces of diseased tissue were cut from the margin of healthy and infected portions using a sterilized scalpel. The tissue pieces were surface sterilized with suitable disinfectant, washed with sterile distilled water and transferred aseptically to sterile nutrient agar (NA) medium. The inoculated plates were incubated at 27 ± 1 °C and observed periodically for bacterial growth. Bacterial colonies developing from the infected tissues were selected based on their colony characteristics and purified by repeated streaking on nutrient agar medium. Pure cultures were maintained on NA slants and used for further studies. The bacterial isolate obtained from diseased fruit and stem tissues was identified as *Pectobacterium* sp. based on its cultural, morphological and biochemical characteristics. A fresh 48-hour-old bacterial broth culture was prepared and used for evaluating the *in-vitro* efficacy of chemicals and antibiotics.

2.2 In-vitro evaluation of chemicals and antibiotics

The efficacy of selected chemicals and antibiotics against *Pectobacterium* sp. was evaluated by the paper disc method described by Thornberry (1950) [14]. Nutrient agar medium was prepared, sterilized in an autoclave and poured into sterile Petri plates. After solidification, each plate was inoculated with 1 ml of 48-hour-old bacterial broth culture and spread uniformly over the surface of the medium using a sterile spreader under aseptic conditions.

For chemical evaluation, eight chemicals, namely hydrogen peroxide, sodium hypochlorite, ammonium bicarbonate, lactic acid, citric acid, copper sulphate, copper oxychloride and copper hydroxide, were tested at 1 and 2 percent concentrations.

For antibiotic evaluation, eight treatments consisting of streptomycin, erythromycin, chloramphenicol, ciprofloxacin, tetracycline, amoxycillin, streptomycin + copper oxychloride and streptomycin + copper hydroxide were tested at 250 and 500 ppm concentrations.

Sterile Whatman No. 1 filter paper discs of 5 mm diameter were separately soaked in the respective treatment solutions for 5 minutes. Two discs representing the lower and higher concentrations of each treatment were placed on opposite sides of each inoculated Petri plate. Three replications were maintained for each treatment. Discs soaked in sterile distilled water were used as the untreated control. The plates were incubated at 27 ± 1 °C for 48-72 hours.

After incubation, the antibacterial activity of each treatment was assessed by measuring the diameter of the clear zone of inhibition formed around the paper discs. The zone of inhibition was measured in millimetres and the mean values of three replications were recorded.

3. Results

3.1 In-vitro efficacy of chemicals

Eight chemicals were evaluated at 1 and 2 percent concentrations against *Pectobacterium* sp. All the chemicals exhibited antibacterial activity, whereas no inhibition was observed in the untreated control.

At 1 percent concentration, copper sulphate recorded the highest inhibition zone of 14.85 mm. It was followed by copper oxychloride (13.20 mm), copper hydroxide (12.40 mm) and ammonium bicarbonate (10.90 mm). Citric acid and sodium hypochlorite produced inhibition zones of 8.36 and 8.15 mm, respectively. Hydrogen peroxide and lactic acid recorded comparatively lower inhibition zones of 7.11 and 6.14 mm, respectively.

At 2 percent concentration, copper hydroxide recorded the maximum inhibition zone of 17.42 mm. Copper oxychloride, ammonium bicarbonate and sodium hypochlorite recorded inhibition zones of 14.96, 14.68 and 14.62 mm, respectively. Copper sulphate and citric acid produced inhibition zones of 12.62 and 12.43 mm, respectively. Hydrogen peroxide and lactic acid showed comparatively lower activity, with inhibition zones of 10.41 and 8.36 mm, respectively. The untreated control did not show any inhibition zone at either concentration.

Table 1: *in vitro* efficacy of chemicals against *Pectobacterium* sp. causing bacterial soft rot of dragon fruit

Tr. No.	Treatments	Inhibition Zone (mm) at 1% Conc.	Inhibition Zone (mm) at 2% Conc.
T ₁	Hydrogen peroxide	7.11	10.41
T ₂	Sodium hypochlorite	8.15	14.62
T ₃	Ammonium bicarbonate	10.90	14.68
T ₄	Lactic acid	6.14	8.36
T ₅	Citric acid	8.36	12.43
T ₆	Copper sulphate	14.85	12.62
T ₇	Copper Oxychloride	13.20	14.96
T ₈	Copper hydroxide	12.40	17.42
T ₉	Control	0.00	0.00
	S. E.±	0.46	0.57
	C.D.(P = 0.01)	1.88	2.33

All the chemicals tested showed inhibition against *Pectobacterium* sp., but their effectiveness differed with treatment and concentration. At 1 percent, copper sulphate gave the highest inhibition zone (14.85 mm), followed by copper oxychloride (13.20 mm) and copper hydroxide (12.40 mm). At 2 percent, copper hydroxide was the best treatment (17.42 mm), followed by copper oxychloride (14.96 mm) and ammonium bicarbonate (14.68 mm) and sodium hypochlorite (14.62 mm). Similar results were reported by Thiagarajan (2016) ^[13], who found copper hydroxide effective against bacterial soft rot. In another study, Ashwini *et al.*, (2022) ^[1] found that 3% sodium hypochlorite gave the highest inhibition zone (12.17 mm), followed by ammonium bicarbonate (11.70 mm), sodium bicarbonate (9.50 mm) and acetic acid (6.70 mm) against *Pectobacterium carotovorum*. Pavitra *et al.*, (2025) ^[9] reported copper oxychloride as the best chemical against *P. carotovorum* subsp. *carotovorum*. Yaganza *et al.*, (2009) ^[15] also reported that different salts could inhibit the growth of *Pectobacterium* spp.



Note: In each Petri plate, the inner paper disc represents the lower concentration (1%), whereas the outer paper disc represents the higher concentration (2%) of the respective chemical.

Plate 1: *in vitro* efficacy of chemicals against *Pectobacterium* sp. causing bacterial soft rot of dragon fruit.

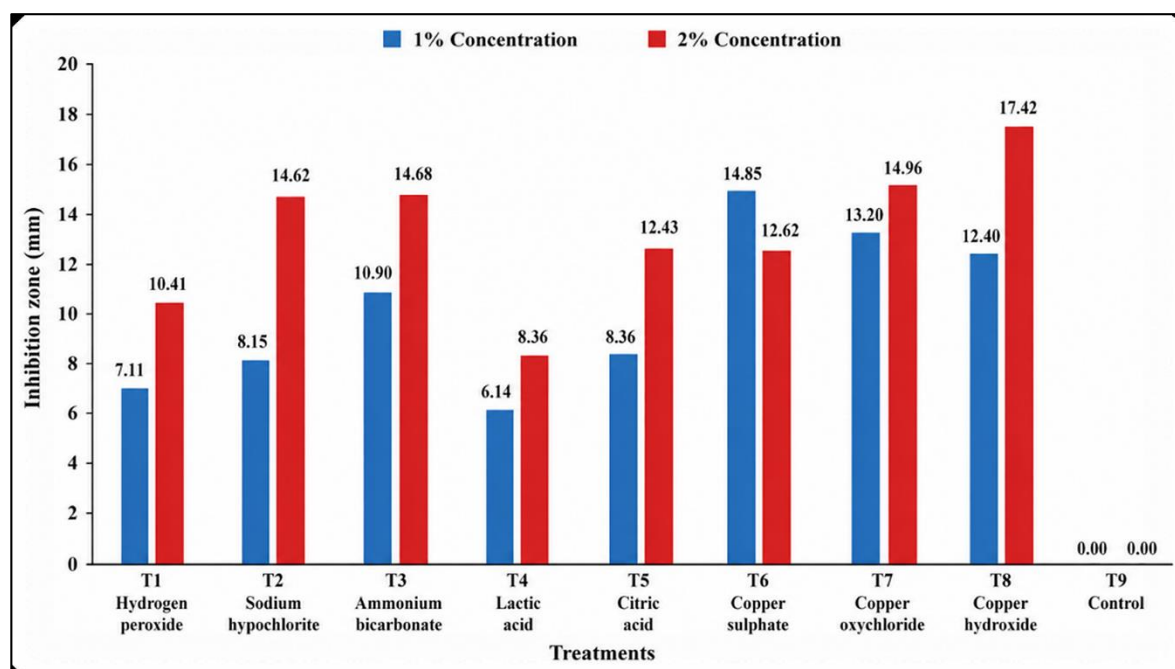


Fig 1: *in vitro* efficacy of chemicals against *Pectobacterium* sp. causing bacterial soft rot of dragon fruit.

Sodium hypochlorite showed better activity at 2 percent (14.62 mm) than at 1 percent (8.15 mm). Hong *et al.*, (2021) ^[4] reported that sodium hypochlorite reduced soft rot caused by *P. carotovorum*. Most chemicals showed better inhibition at 2 percent, but copper sulphate gave a lower zone at 2 percent (12.62 mm) than at 1 percent (14.85 mm). Hydrogen peroxide, citric acid and lactic acid were less effective. The difference in activity may be due to the sensitivity of the bacterial isolate and the nature of the chemicals. Overall, copper hydroxide, copper oxychloride, ammonium bicarbonate and sodium hypochlorite were the better treatments under *in-vitro* conditions.

3.2 *In-vitro* efficacy of antibiotics

Eight antibiotic treatments were evaluated at 250 and 500 ppm. All the antibiotics produced inhibition zones against *Pectobacterium* sp., although their effectiveness varied considerably.

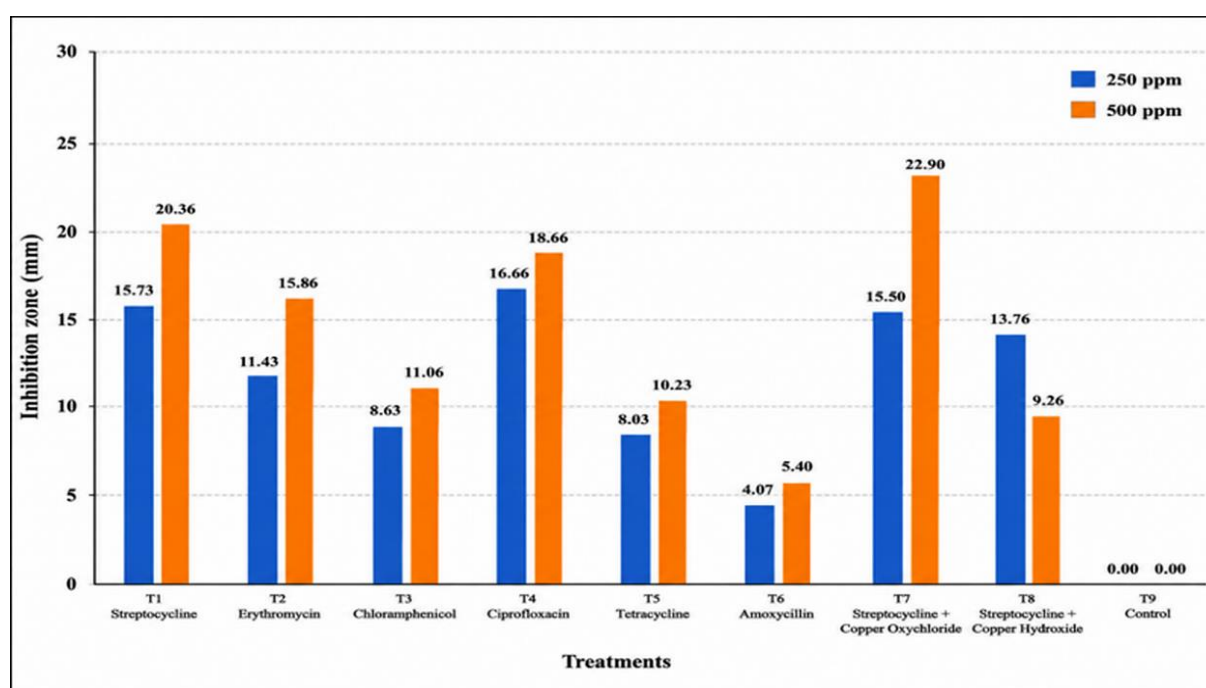
At 250 ppm, ciprofloxacin recorded the highest inhibition zone of 16.66 mm, followed by streptomycin (15.73 mm), streptomycin + copper oxychloride (15.50 mm) and streptomycin + copper hydroxide (13.76 mm). Erythromycin, chloramphenicol, tetracycline and amoxycillin produced inhibition zones of 11.43, 8.63, 8.03 and 4.07 mm, respectively.

Table 2: *In vitro* efficacy of antibiotics against *Pectobacterium* sp. causing bacterial soft rot of dragon fruit.

Tr. No.	Treatments	Inhibition zone (mm) at concentration 250 ppm	Inhibition zone (mm) at concentration 500 ppm
T ₁	Streptocycline	15.73	20.36
T ₂	Erythromycin	11.43	15.86
T ₃	Chloramphenicol	8.63	11.06
T ₄	Ciprofloxacin	16.66	18.66
T ₅	Tetracycline	8.03	10.23
T ₆	Amoxycillin	4.07	5.4
T ₇	Streptocycline + Copper Oxychloride	15.50	22.9
T ₈	Streptocycline + Copper Hydroxide	13.76	9.26
T ₉	Control	0.00	0.00
	S. E.±	0.20	0.35
	C.D.(P = 0.01)	0.81	1.45



Note: In each Petri plate, the inner paper disc represents the lower concentration (250 ppm), whereas the outer paper disc represents the higher concentration (500 ppm) of the respective antibiotic

Plate 2: *In vitro* efficacy of antibiotics against *Pectobacterium* sp. causing bacterial soft rot of dragon fruit.**Fig 2:** *In vitro* efficacy of antibiotics against *Pectobacterium* sp. causing bacterial soft rot of dragon fruit.

At 500 ppm, streptocycline + copper oxychloride recorded the highest inhibition zone of 22.90 mm, followed by streptocycline (20.36 mm) and ciprofloxacin (18.66 mm). Erythromycin recorded 15.86 mm, followed by chloramphenicol (11.06 mm), tetracycline (10.23 mm), streptocycline + copper hydroxide (9.26 mm) and amoxycillin (5.40 mm). No inhibition zone was observed in the untreated control.

At 250 ppm, ciprofloxacin recorded the highest inhibition zone (16.66 mm), followed by streptocycline (15.73 mm) and streptocycline + copper oxychloride (15.50 mm). At 500 ppm, streptocycline + copper oxychloride was the most effective treatment (22.90 mm), followed by streptocycline (20.36 mm) and ciprofloxacin (18.66 mm). Erythromycin, chloramphenicol and tetracycline showed moderate activity, while amoxycillin was the least effective. Sang *et al.* (2015)^[10] reported good activity of streptomycin against *P. carotovorum*. Pavitra *et al.* (2025)^[9] also reported good activity of streptomycin sulphate against *P. carotovorum* subsp. *carotovorum*. Thammaiah *et al.* (2005) recorded 24.00 mm inhibition with streptocycline + copper oxychloride against *Erwinia* sp., which is close to the result obtained in the present study.

Most antibiotics showed higher inhibition at 500 ppm than at 250 ppm. However, streptocycline + copper hydroxide showed lower inhibition at 500 ppm (9.26 mm) than at 250 ppm (13.76 mm). The difference may be due to the response of the bacterial isolate or the interaction between the antibiotic and copper compound. Shen *et al.* (2025)^[11] reported strong activity of streptomycin against *P. carotovorum* and also highlighted the problem of streptomycin resistance. Therefore, the effective treatments found in the present study, particularly streptocycline + copper oxychloride, should be further tested under field conditions before practical recommendation.

Conclusion

The present study confirmed the occurrence of bacterial soft rot of dragon fruit caused by *Pectobacterium* sp. In the *in-vitro* chemical evaluation, all the tested chemicals showed inhibitory activity against *Pectobacterium* sp. Copper hydroxide recorded the highest inhibition zone of 17.42 mm at 2%, followed by copper oxychloride (14.96 mm), ammonium bicarbonate (14.68 mm) and sodium hypochlorite (14.62 mm). Among the antibiotics, streptocycline + copper oxychloride recorded the highest inhibition zone of 22.90 mm at 500 ppm, followed by streptocycline (20.36 mm) and ciprofloxacin (18.66 mm). Therefore, copper hydroxide, copper oxychloride, sodium hypochlorite, streptocycline and streptocycline + copper oxychloride showed good *in vitro* activity against *Pectobacterium* sp. These treatments may be useful for further field evaluation and management of bacterial soft rot of dragon fruit.

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