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In-vitro evaluation of different fungicides and antibiotics against bacterial pathogen *Xanthomonas campestris* pv. *campestris* causing black rot of cabbage

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Abstract
Cabbage (*Brassica* family) is a vital winter vegetable, globally valued for its nutritional content and health benefits. Black rot, caused by *Xanthomonas campestris* pv. *campestris* (*Xcc*), is a prevalent and devastating disease affecting cabbage, leading to substantial yield losses. This study aimed to evaluate the inhibitory effect of eleven different fungicides and antibiotics against *Xcc* under *in-vitro* conditions using an agar well method with three different concentrations. Results indicated that all tested fungicides and antibiotics significantly reduced *Xcc* growth compared to the control. The maximum growth inhibition was recorded in streptomycin sulphate 90% + tetracycline hydrochloride 10% SP mixed with copper oxychloride 50% WP at all three concentrations tested. Another effective treatment was the combination of Streptomycin sulphate 90% + tetracycline hydrochloride 10% & Bordeaux mixture 1%. These findings offer valuable insights for farmers and researchers seeking to combat this destructive disease in cabbage cultivation.

Keywords: Black rot, cabbage, inhibition, fungicides, antibiotics, *X. campestris* pv. *campestris*

Introduction
Cabbage (*Brassica oleracea* var. *capitata* L.) is a leafy winter vegetable from the *Brassica* family, widely consumed raw or cooked in various ways (Hanif *et al.*, 2006) [5]. It is low in calories, high in fiber, and contains calcium, iron, vitamins A, C, and beneficial enzymes along with that, a key phytochemical, glucosinolate, present in cabbage is believed to protect humans against cancer (Sarikamis, 2009, Gupta *et al*., 2013) [9, 4]. It is cultivated worldwide and India ranks second in production after China. Being one of the highest cabbage producing country, it is necessary to manage various stresses and diseases which impacts the commercial cabbage cultivation to meet its demand as it is a dominant vegetable in the human diet. The crop is infected by a variety of pathogens both in the nursery and the field conditions (Elsisi, 2017) [3]. Amongst different diseases affecting cabbage, black rot caused by *Xanthomonas campestris* pv. *campestris* (*Xcc*) is very common and highly destructive. It can cause huge yield losses by premature defoliation and reducing the quality of heads in cabbage. The disease has a wide geographical distribution causing yield (50-65%) and quality loss of crucifers based on cultivar susceptibility (Williams, 1980; Ignatov *et al.*, 1999; Kashyap and Dhiman, 2010; Dhar and Singh, 2014) [11, 6, 7, 2]. The bacterium causes 10–50 per cent damages to Cole crops if favourable environmental conditions occur. In view of such potential crop losses and considering the economic significance of the disease, *in vitro* evaluation studies were conducted against the pathogen with different fungicides and agrochemicals. The treatments found best in the experiment can be further employed in integrated disease management.

Materials and Methods
The experiment was conducted at the PG research laboratory, Department of Plant Pathology, BACA, AAU, Anand, Gujarat, India. The inhibitory effect of eleven different fungicides and antibiotics was evaluated against *X. campestris* pv. *campestris in-vitro* by using an agar diffusion method (Monteiro *et al.*, 2005) [8] with three different concentrations and three repetitions. A completely randomized design (CRD) was used as an experimental design. The details of different treatments with their concentration is mentioned here under:

Treatment details

Tr. No.	Treatments	Concentrations (ppm)		
T_{1-3}	Validamycin 3% L	100	200	300
T_{4-6}	Aureofungin 46.15% w/v SP	100	200	300
T_{7-9}	Kasugamycin 3% SL	100	200	300
T_{10-12}	Copper oxychloride 50% WP	100	200	300
T_{13-15}	Copper hydroxide 77% WP	100	200	300
T_{16-18}	Hexaconazole 5% EC	100	200	300
T_{19-21}	Hexaconazole 5% + validamycin 2.5% SC	100	200	300
T_{22-24}	Streptomycin sulphate 90% + tetracycline hydrochloride10% SP	100	200	300
T_{25-27}	Kasugamycin 5% + copper oxychloride 45% WP	100	200	300
T_{28-30}	Streptomycin sulphate 90% + tetracycline hydrochloride 10% SP & copper oxychloride 50 WP (manually mix)	100 + 1000	200 + 2000	300 + 3000
T_{31-33}	Streptomycin sulphate 90% + tetracycline hydrochloride 10% & Bordeaux mixture 1% (manually mix)	100 + 1000	200 + 2000	300 + 3000
T_{34}	Control (Test bacterium only)	--	--	--

Methodology

Three days old bacterial culture of *Xcc* (1×10^8 cfu) were maintained in nutrient broth. Molten nutrient agar was inoculated with bacterial culture @ 1 ml/100 ml of NA. Nutrient agar was poured into the sterilized Petri plates and allowed to solidify. A well (5 mm in diameter) was made by piercing the nutrient agar with a sterilized cork borer on the corner of the plate in four directions by leaving a distance of 1 cm from the periphery of the plates. Each well was poured with 50 µl of various agrochemicals at different concentrations. Three repetitions were kept for each concentration of respective treatments. The growth of test bacteria on non-treated NA media, served as a control. Plates were incubated at 27 ºC for 36 hrs. After the 36 hrs. of incubation, observations on inhibition zone of test bacteria (mm), growth inhibition (%) were recorded.

Results and Discussion

All the fungicides and antibiotics significantly reduced the growth of *Xcc* as compared to control. The data are presented in Table 1 and depicted in Fig.1.

The highest growth inhibition of 21.90, 25.50, 27.07 percent and inhibition zone of 19.71, 22.95 and 24.36 mm was recorded in streptomycin sulphate 90% + tetracycline hydrochloride 10% SP mixed with copper oxychloride 50% WP at 100+1000, 200+2000 and 300+3000 ppm, respectively. Next better treatment in order was streptomycin sulphate 90% + tetracycline hydrochloride 10% and Bordeaux mixture 1% which recorded inhibition zone of 17.66, 20.52 and 22.40 mm in all the three concentrations, respectively.

The lowest zone of inhibition was recorded in Kasugamycin 3% SL followed by Hexaconazole 5% EC with all three concentrations.

Table 1: Efficacy of fungicides and antibiotics against *X. campestris* pv. *campestris* under *in-vitro* conditions

Tr. No.	Treatments	Concentration (ppm)	Zone of inhibition (mm)	Growth inhibition (%)
T_1	Validamycin 3% L	100	6.92 ^{g}	7.70
T_2		200	9.25 ^{g}	10.27
T_3		300	10.79 ^{f}	11.99
T_4	Aureofungin 46.15% w/v SP	100	3.22 ^{i}	3.72
T_5		200	3.87 ^{i}	4.30
T_6		300	4.78 ^{i}	5.31
T_7	Kasugamycin 3% SL	100	4.58 ^{h}	5.09
T_8		200	5.55 ^{h}	6.16
T_9		300	7.82 ^{g}	8.69
T_{10}	Copper oxychloride 50% WP	100	11.93^{d}	13.26
T_{11}		200	14.45 ^{c}	16.06
T_{12}		300	15.72 ^{c}	17.46
T_{13}	Copper hydroxide 77% WP	100	9.97 ^{e}	11.08
T_{14}		200	12.60 ^{e}	14.00
T_{15}		300	14.78 ^{d}	16.42
T_{16}	Hexaconazole 5% EC	100	4.61 ^{h}	5.13
T_{17}		200	5.15 ^{h}	5.72
T_{18}		300	6.04 ^{h}	6.71
T_{19}	Hexaconazole 5% + validamycin 2.5% SC	100	12.87 ^{c}	14.30
T_{20}		200	13.62 ^{d}	15.13
T_{21}		300	14.25 ^{d}	15.83
T_{22}	Streptomycin sulphate 90% + tetracycline hydrochloride10% SP	100	12.57 ^{c}	13.96
T_{23}		200	12.94 ^{e}	14.38
T_{24}		300	15.97 ^{c}	17.74
T_{25}	Kasugamycin 5% + copper oxychloride 45% WP	100	9.39 ^{f}	10.43
T_{26}		200	10.91 ^{f}	12.13
T_{27}		300	12.14 ^{e}	13.49

T_{28}	Streptomycin sulphate 90% + tetracycline hydrochloride 10% SP & copper oxychloride 50 WP (Manual mix)	100+ 1000	19.71 [a]	21.90
T_{29}		200+ 2000	22.95 [a]	25.50
T_{30}		300+ 3000	24.36 [a]	27.07
T_{31}	Streptomycin sulphate 90% + tetracycline hydrochloride 10% & Bordeaux mixture 1% (Manual mix)	100+ 1000	17.66 [b]	19.62
T_{32}		200+ 2000	20.52 [b]	22.80
T_{33}		300+ 3000	22.40 [b]	24.89
T_{34}	Control	-	0.00 [j]	0.00
	S.Em. ±	-	0.20	-
	C.D. at 5%	-	Sig.	-
	C.V. (%)	-	3.06	-

Note: Treatment means with the letter/letters in common are not significant by Duncan's new multiple range test (DNMRT) at a 5% level of significance

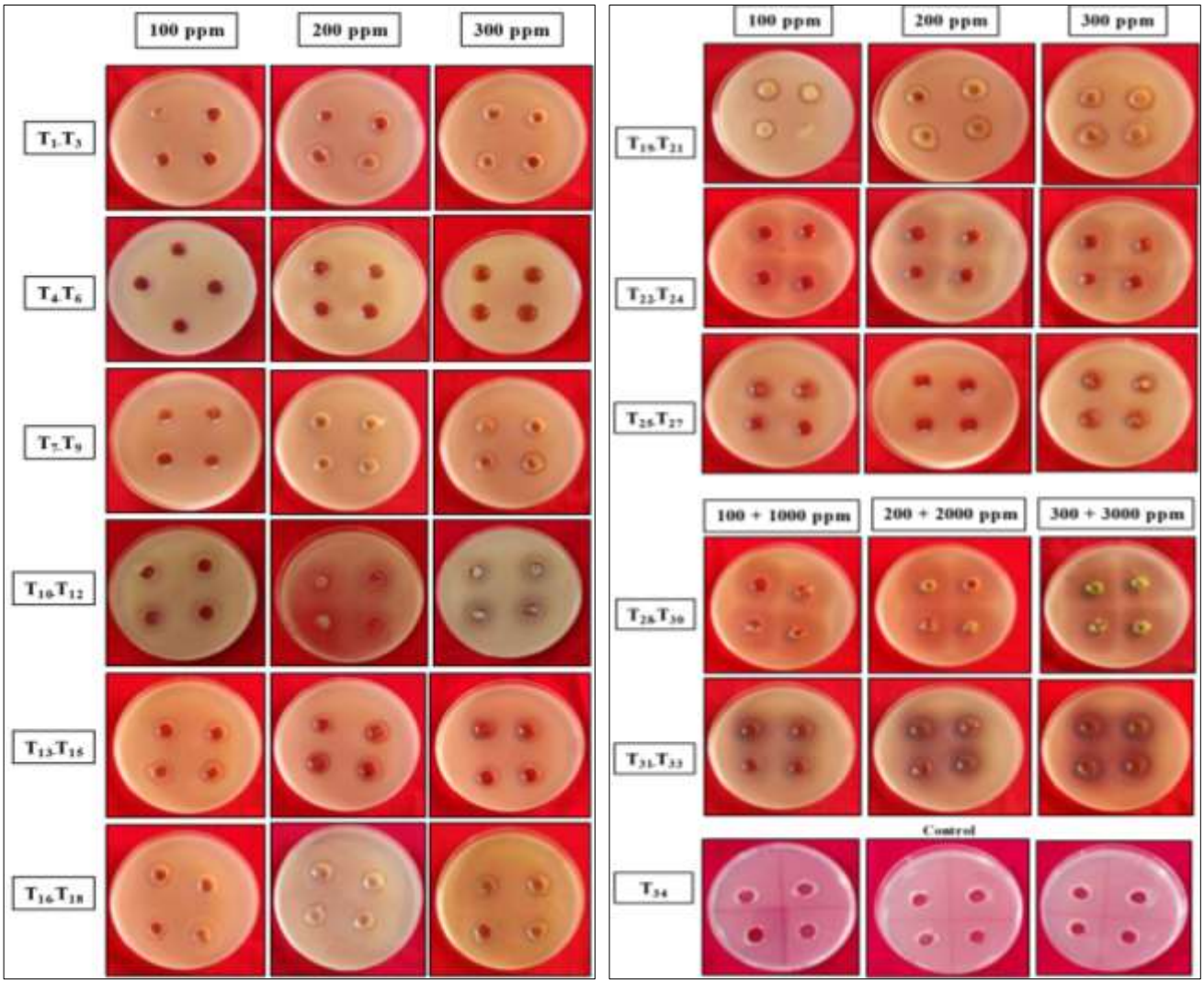

Fig 1: Evaluation of different fungicides and antibiotics against *Xcc* under *in-vitro* conditions

The current findings are also in confirmation with the Basur (2012) [1] and Suthar (2020) [10]. Basur (2012) [1] found streptocycline at 1000 ppm + copper oxychloride at 2000 ppm gave 57.06 percent reduction over control for *Xcc* infection in cabbage.

While, Suthar (2020) [10] found streptomycin sulphate 90% + tetracycline hydrochloride 10% SP mixed with copper oxychloride 50 WP gave 20.28, 22.62 and 26.08 mm zone of inhibition with percent growth inhibition of 22.54, 25.13 and 28.97% at all three concentrations (100, 200 and 300 ppm), respectively against *X. citri* pv. *citri*.

Conclusion

Out of eleven different fungicides and antibiotics tested against *Xcc* at different concentrations using an agar well diffusion technique, the highest growth inhibition of 21.90, 25.50, 27.07 percent and inhibition zone of 19.71 mm, 22.95 mm and 24.36 mm was recorded in Streptomycin sulphate 90% + tetracycline hydrochloride 10% SP mixed with copper oxychloride 50% WP with 100+1000, 200+2000 and 300+3000 ppm respectively. However, Streptomycin sulphate 90% + tetracycline hydrochloride 10% and Bordeaux mixture 1% was found equally effective with inhibition zone of 17.66, 20.52 and 22.40 mm in all three concentrations, respectively.

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