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Bioefficacy of Chemicals against Bacterial Leaf Blight Disease of Rice

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A B S T R A C T

Bacterial leaf blight of rice, caused by *Xanthomonas oryzae* pv. *oryzae* is not only a problem in India but also worldwide. Management using chemicals is undoubtedly quick in action, restricting the severity and spread of the disease. Both *in vitro* and *in vivo* studies were conducted to investigate the inhibitory potential of different chemicals against the bacterial pathogen. Out of eight chemicals comprising both antibiotics and fungicides, Streptomycin sulphate 90% + Tetracycline hydrochloride 10% exhibited highest inhibitory effect in lowest concentration of 100 ppm followed by 2-bromo-2-nitropropane 1,3-diol (15.5%). Streptomycin sulphate 90% + Tetracycline hydrochloride 10% @ 100 ppm proved best in controlling BLB in pot condition with 42.2% disease reduction and 121 % yield increase over control.

Introduction

Rice, the golden cereal, is an important part of daily human food and provides carbohydrates, proteins, minerals and vitamins (Pradhan *et al.*, 2019). With the global population expanding at a rapid rate, it warrants a solution to food scarcity and hunger. To provide food for the billions, the productivity of rice needs to be increased.

But yield losses tend to rise with swelling pest population which is the consequence of changing climate and agroecological systems.

A number of diseases pose threat to the rice industry and bacterial leaf blight (BLB) is a major one which can hamper more than 70% yield (Reddy *et al.*, 1979). BLB is caused by *Xanthomonas oryzae* pv. *oryzae (Xoo)*, which enters the plant system via hydathodes (Nino

et al., 2006). *Xoo* attacks the crop mainly at two stages viz., nursery and tillering. *Kresek* is a symptom associated with wilting of seedlings. Leaf blight occurs mainly from tillering to flowering stage. The intensity and type of blight symptoms vary with varieties, environmental conditions and crop growth stages.

A number of management strategies such as host resistance, biological control and chemical control are employed to overcome the disease. But sometimes, sudden appearance of this disease in the field puzzles the farmer. In this situation, the use of chemicals becomes indispensable for a quick and effective solution. An investigation was conducted in ICAR-NRRI, Cuttack using some antibiotics and fungicides, both in vitro and in vivo to find out the best chemical for management of BLB.

Materials and Methods

Collection, isolation, purification and pathogenicity of the pathogen

Fifty-two disease samples were collected from few states of Eastern India. The pathogen was isolated by ooze method (Kotasthane, 2003) and purified on modified Wakimoto's agar (MWA).

Pathogenicity tests were performed on Taichung Native1 plants (BLB susceptible cultivar) by leaf clipping (Kauffman *et al.*, 1973). The isolates were subjected to virulence profiling using differentials and a representative isolate from the most virulent pathotype was used for this study.

Antimicrobial assay under *in vitro* conditions

The entire procedure of agar disc diffusion (Baeur *et al.*, 1966) was done aseptically. Stock solutions of 10,000 ppm were prepared for each chemical using sterile distilled water. For each chemical, seven concentrations consisting of 5000, 2500, 1000, 750, 500, 250 and 100 ppm were prepared employing serial dilution. A homogenous bacterial suspension was prepared from three days- old culture and sterile distilled water.

A lawn of inoculum was spread onto solidified MWA plates and allowed to dry for 30 minutes. Sterile disc (HiMedia Laboratories) of 6 mm diameter impregnated with 30 µl of chemical was placed at the centre of the plate with inoculum lawn and incubated. The experiment was conducted with three replications per treatment. After 72 hours, the zone of inhibition (excluding the diameter of the disc) was recorded and percent inhibition was calculated as follows:

$$\%\ inhibition = \frac{Diameter\ of\ the\ zone\ of\ inhibition\ \times 100}{Diameter\ of\ the\ petriplate}$$

Antimicrobial assay under *in vivo* conditions

From the *in vitro* experiment, two lower concentrations of all the test chemicals showing inhibitory effect were chosen. An experiment with a total of 17 treatments including control and three replications each was designed. TN1 cultivar was used for the trial. Standard agronomic practices were followed. Disease was clip-inoculated to the plants at 45 days after sowing. After 10 days, a foliar spray of the chemicals was given while the control was sprayed with sterile water only. The disease score (IRRI,1996) was recorded after 21 days of inoculation. Percent disease index was computed as follows:

$$\text{PDI}\ (\%) = \frac{Sum\ of\ all\ blight\ scores \times 100}{Number\ of\ leaves\ scored \times Maximum\ rating}$$

Results and Discussion

Antimicrobial assay under *in vitro* conditions

As presented in Table 1, the sensitivity of the *Xoo* isolate had a varied response to the different chemicals used as well as their concentrations. Among all, a maximum of 5.5 cm zone of inhibition was recorded for chloramphenicol at 5000 ppm.

But chloramphenicol was isolate specific in inhibitory effect (Khan *et al.,* 2012). Copper oxychloride and copper hydroxide were totally ineffective at 500, 250 and 100 ppm concentrations.

The former chemical was equally effective as Captan at 5000 and 1000 ppm strength. Interestingly, inhibitions by chloramphenicol were at par with 2-bromo-2-nitropropane 1,3-diol at 500 ppm and with streptomycin sulphate at 100 ppm respectively.

A ranking with a descending trend in the efficacy of chloramphenicol, 2-bromo-2-nitropropane 1,3-diol, streptocycline and streptomycin sulphate was observed from 5000 to 1000 ppm.

However, at the two lower concentrations (250 and 100 ppm), streptocycline was the most effective of all, followed by chloramphenicol, 2-bromo-2-nitropropane 1,3-diol and streptomycin sulphate. Streptocycline had promising effect to check *Xoo* (Mahto *et al.,* 1988).

The chemical, 2-bromo-2-nitropropane 1,3-diol produced excellent control over the pathogen under *in vitro* conditions (Praveen *et al.,* 2019).

Surprisingly, all the chemicals and their concentrations failed to exhibit any uniform trend in inhibition of the pathogen. It partially corroborated with the previous studies, where increase in inhibition with rise in concentration followed a regular trend (Khan *et al.,* 2012 and Ashrafuzzaman, 1987).

This indicated the presence of a specific type of interaction of the microbe with varying chemicals and concentrations.

Antimicrobial assay under *in vivo* conditions

At both the concentrations, chloramphenicol, 2-bromo-2-nitropropane 1,3-diol, streptocycline and streptomycin sulphate were able to restrict the disease by 19-25% over control (Table 2).

A maximum yield (26.36 g/pot) was attained using streptocycline spray @ 250 ppm, followed by chloramphenicol (24.80 g/pot) and 2-bromo-2-nitropropane 1,3-diol (24.10 g/pot) at the same concentration.

Satisfactory disease control and yield was experienced when 2-bromo-2-nitropropane 1,3-diol was used against the disease (Pramesh *et al.,* 2017).

Eight treatments belonging to Mancozeb, Captan, copper hydroxide and copper oxychloride were ineffective in restricting the disease.

However, they produced equal yield among themselves, but superior to control. Treatment with copper oxychloride produced fair yield performance in rice against BLB (Shahbaz *et al.,* 2016 and Chaudhary *et al.,* 2012).

Table.1 Evaluation of *in vitro* efficacy of chemicals against *Xanthomonas oryzae* pv. *Oryzae*

Treatment	Chemical name	Trade name	Concentration (ppm)													
			5000		2500		1000		750		500		250		100	
			Mean zone of inhibition (cm)	% inhibition	Mean zone of inhibition (cm)	% inhibition	Mean zone of inhibition (cm)	% inhibition	Mean zone of inhibition (cm)	% inhibition	Mean zone of inhibition (cm)	% inhibition	Mean zone of inhibition (cm)	% inhibition	Mean zone of inhibition (cm)	% inhibition
T1	Chloramphenicol 500mg	Chloramphenicol	5.5 (2.5)*	65.5	5.1 (2.4)	60.3	4.3 (2.2)	51.2	4.1 (2.1)	48.4	3.2 (1.9)	38.1	2.5 (1.7)	30.2	1.1 (1.3)	13.5
T2	2-bromo-2-nitropropane 1,3-diol	Bactinash-200	5.2 (2.4)	61.9	4.7 (2.3)	56.0	3.8 (2.1)	45.3	3.7 (2.1)	44.4	3.2 (1.9)	38.1	2.2 (1.7)	26.6	1.3 (1.3)	15.5
T3	Streptomycin sulphate 90% + Tetracycline hydrochloride 10%	Streptocycline	4.4 (2.2)	52.4	3.7 (2.1)	44.4	3.4 (2.0)	40.5	3.0 (1.9)	36.1	3.0 (1.9)	35.7	2.7 (1.8)	31.8	1.6 (1.5)	19.4
T4	Mancozeb 75% WP	Mancozeb	3.0 (1.9)	36.1	2.8 (1.8)	33.7	2.4 (1.7)	29.0	2.0 (1.6)	23.8	1.6 (1.5)	19.4	1.3 (1.3)	15.5	1.0 (1.2)	12.3
T5	Captan 50% WP	Captan	1.8 (1.5)	21.0	1.3 (1.3)	15.5	1.2 (1.3)	14.3	0.9 (1.2)	11.1	0.8 (1.1)	9.5	0.8 (1.1)	9.1	0.6 (1.0)	7.1
T6	Copper hydroxide 77% WP	Kocide	1.6 (1.4)	18.7	1.0 (1.2)	11.9	0.7 (1.1)	8.7	0.6 (1.0)	6.8	0.0 (0.7)	0.0	0.0 (0.7)	0.0	0.0 (0.7)	0.0
T7	Copper oxychloride 50% WP	Blitox50	1.8 (1.5)	21.0	1.5 (1.4)	17.9	1.2 (1.4)	14.7	1.1 (1.3)	13.1	0.0 (0.7)	0.0	0.0 (0.7)	0.0	0.0 (0.7)	0.0
T8	Streptomycin sulphate	Streptomycin sulphate	3.5 (2.0)	41.7	3.3 (2.0)	39.7	3.2 (1.9)	38.5	3.2 (1.9)	38.1	3.0 (1.9)	35.7	1.8 (1.5)	21.8	1.2 (1.3)	13.9
T9	Control	Control	0.0 (0.7)		0.0 (0.7)		0.0 (0.7)		0.0 (0.7)		0.0 (0.7)		0.0 (0.7)		0.0 (0.7)	
	SE(m)±		0.01		0.01		0.01		0.01		0.01		0.01		0.01	
	CD (0.05)		0.03		0.03		0.03		0.03		0.02		0.03		0.03	

* Data in parenthesis represent √ (x + 0.5) transformed values

Table.2 Evaluation of *in vivo* efficacy of chemicals against *Xanthomonas oryzae* pv. *Oryzae*

Treatment	Chemical name	Trade name	Mean PDI (%)	% reduction over control	Mean yield (g/pot)	% increase over control
T1	Chloramphenicol 500mg	Chloramphenicol @ 250ppm	77.8(61.9)*	22.2	24.8	127.3
T2	Chloramphenicol 500mg	Chloramphenicol @ 100ppm	77.8 (61.9)	22.2	21.6	97.7
T3	2-bromo-2-nitropropane 1,3-diol	Bactinash @ 250ppm	77.8 (61.9)	22.2	24.1	120.9
T4	2-bromo-2-nitropropane 1,3-diol	Bactinash @ 100ppm	77.8 (61.9)	22.2	21.3	95.3
T5	Streptomycin sulphate 90% + Tetracycline hydrochloride 10%	Streptocycline @ 250ppm	74.8 (60.0)	25.2	26.3	141.6
T6	Streptomycin sulphate 90% + Tetracycline hydrochloride 10%	Streptocycline @ 100ppm	77.8 (61.9)	22.2	24.1	121
T7	Mancozeb 75% WP	Mancozeb @ 250ppm	100.0(90.0)	0	15.8	44.6
T8	Mancozeb 75% WP	Mancozeb @ 100ppm	100.0(90.0)	0	15.2	39.4
T9	Captan 50% WP	Captan @ 250ppm	100.0(90.0)	0	15.2	39
T10	Captan 50% WP	Captan @ 100ppm	100.0(90.0)	0	15.1	37.9
T11	Copper hydroxide 77% WP	Kocide @ 1000ppm	86.7 (68.6)	13.3	16.8	54.2
T12	Copper hydroxide 77% WP	Kocide @ 750ppm	95.6 (80.2)	4.4	16.6	51.9
T13	Copper oxychloride 50% WP	Blitox50 @ 1000ppm	82.2 (65.2)	17.8	17.7	62.1
T14	Copper oxychloride 50% WP	Blitox50 @ 750ppm	91.1 (72.6)	8.9	16.8	53.7
T15	Streptomycin sulphate	Streptomycin Sulphate @ 250ppm	77.8 (61.9)	22.2	22.5	106.4
T16	Streptomycin sulphate	Streptomycin Sulphate @ 100ppm	80.7 (64.0)	19.3	18.3	67.5
T17		Control	100.0 (90.0)		10.9	
		SE(m)±	1.44		0.99	
		C.D. (0.05)	4.15		2.87	

*Data in parenthesis indicate arcsine transformed values

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