Madras Agric. J., 99 (7-9): 592-596, September 2012
https://doi.org/10.29321/MAJ.10.100149

Efficacy of Antibiotics and Biorational Pesticides against Bacterial Blight of Paddy

P.R. Thimmegowda[1*], Arun Sataraddi[2], D.S. Ambika[1], P.S. Prasad[1] and M. Chandrashekar[3]

[1]Department of Plant Pathology, College of Agriculture, G.K.V.K, UAS, Bengaluru-560 065,
[2] Department of Plant Pathology, College of Agriculture, UASD, Bijapur,
[3]Department of Agricultural Microbiology, College of Agriculture, G.K.V.K, UAS, Bengaluru-560 065

In rice, streptocycline at 1000 ppm was effective against bacterial blight under *in vitro* evaluation, with maximum inhibition zone (25.0mm), followed by streptocycline + Copper sulphate 1000 ppm (24.33mm). Under field studies, streptocycline + copper oxychloride was found best with least per cent disease index. Highest grain yield returns were recorded in streptocycline + copper oxychloride treatment @ 0.025 % + 0.1 per cent followed by bactrinashak @ 0.04 and 0.03 per cent.

Key words: Bacterial blight of rice, *Xanthomonas oryzae* pv. *oryzae,* management, antibiotics and biorational pesticides.

Rice is the most important cereal crop in south east Asia. In Asia alone world's 96 per cent of rice is produced. Bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* (Ishiyama, 1922; Swings *et al* . 1990) is the major constraint for production all over the world causing 20-30 per cent yield losses (Ou, 1985; Islam and Bora, 1998 and Kumar *et al*.1999). Cultivation of high yielding varieties, intensive farming practices and application of high dose of nitrogen frequently aggravate the severity of BB. This leads to the reduced yield and grain quality. A very limited success is achieved in the control of this disease so far due to its fast spreading nature.

The severity and significance of damage caused by infection necessitated development of strategies to control and manage the disease so as to reduce crop loss and to avert an epidemic. Though the use of Bordeaux mixture, antibiotics, copper and mercurial compounds were resorted to in the early fifties, environmentally safe and stable chemical control agents rendering control at low concentrations are yet to be developed. Keeping this in view the present investigations were under taken to study management strategies against bacterial blight to reduce yield losses.

Material and Methods

An immunomodulator, Bactrinashak, antibiotics like streptocycline, tetracycline, cyclohexamide, Agrimycin 100, Copper oxychloride (COC), combination of Streptocycline + copper oxychloride and Streptocycline + Copper sulphate were tested at 250, 500, 750 and 1000 ppm under laboratory condition by paper disc or inhibition zone method against *Xanthomonas oryzae* pv. *oryzae* to know their efficacy in inhibiting the growth of bacteria. A heavy suspension of *Xanthomonas oryzae* pv. *oryzae* (7x 10_8 cfu/ml) was mixed with molten potato sucrose agar (PSA) contained in 500 ml Erlenmeyer flask, so as to get a thick growth of bacteria on the medium. The seeded medium was poured in sterilized petri plates and allowed to solidify.

Previously sterilized filter paper discs (Whatman No. 42) measuring five mm diameter were soaked in different antibiotic solutions for five minutes and transferred on to the surface of the seeded medium contained in petridishes. Filter paper discs dipped in sterile water served as check.The inoculated plates were placed first at 4 to 5_0C in the refrigerator for 3 to 4 hrs to allow the chemicals to diffuse into the medium.Then the plates were incubated at 27 or 28_0 ± 1_0C for 48 hrs. Observations were recorded after 48 hr on the zone of inhibition produced around the filter paper discs by measuring the diameter of the inhibition using antibiotic inhibition zone scale.

Chemicals effective under *in vitro* were tested in farmer's field, where the experiment was laid out in randomized complete block design (RCBD) during the *kharif* season. The variety Sonamahsuri was grown and sprayed with different bio- rational pesticides, botanicals and bio-agent (Table 2). Two sprays were given one spray at 30 days after transplanting (DAT) as a prophylactic spray and second spray at 60 DAT. In control plot no spray was given. Disease incidence was recorded at 60 DAT and 90 DAT. The experiment included seven treatments and a check with three replications.

In each plot, 10 rice plants were selected diagonally representing the entire plot and labeled

*[1]Corresponding author email: tgowda123@gmail.com

with tags, in each plant five leaves were scored by using the 0-9 scale (Anonymous., 1996) as described below. 0= No affected area, 1= 1-5 per cent affected area, 3 = 6-12 per cent affected area, 5 = 13-25 per cent affected area, 7 = 26-50 per cent affected area, 9 = 51-100 per cent affected area, Final observation was taken on 90_{th} day after transplanting. Per cent disease index (PDI) was worked out by using the formula given by Singh (2002).

At the time of harvesting yield returns due to chemical spray was calculated for each treatment over control by taking into consideration the cost incurred on chemicals, labour and additional yield obtained in terms of money over untreated check. Gross return (GR) = Total profit earned by selling the produce i.e., yield x selling rate of Paddy Net return (NR) = [Gross return - (cost of chemicals for two sprays + labour cost for spraying)]

Phenol Estimation

Leaf samples were collected a day after each spray. Leaf material was extracted in ethanol as per the procedure followed by Jaypal and Mahadevan (1968) and clarified with saturated solution of lead acetate. The excess lead acetate was precipitated by the addition of sufficient quantity of saturated solution of sodium hydrogen orthophosphate. The precipitate was re-separated by filtering the alcohol extract through Whatman No.1 filter paper and the filtrate was made up to a known volume with 80 per cent alcohol. Phenols and ortho-dihydroxy phenols were estimated in the alcohol extract of fresh leaves.

Estimation of total phenols: Estimation of total phenols present in plant samples was carried out by Folin -Ciocalteau Reagent method and Arnow's method was used to estimate ortho-dihydroxy phenols.

Results and Discussion

In vitro evaluation of antibiotics

The differences among the treatments and concentrations were found to be statistically significant except between Streptocycline and Streptocycline + Copper sulphate. There was significant difference between the concentrations of treatments. Among them, streptocycline was significantly superior from the rest of the chemicals with mean inhibition zone of 22.83 mm (Table. 1) in all the tested concentrations, followed by streptomycin sulphate, streptocycline + Copper sulphate, Streptocycline + Copper oxychloride, Agrimycin 100 and Cyclohexamide (21.91 mm, 21.58 mm, 20.33 mm, 16.25 mm and 12.58 mm respectively). Copper oxychloride exhibited zero inhibition zone showing ineffectiveness. Among concentrations, 1000 ppm recorded the mean maximum inhibition zone (14.48 mm) followed by 750 and 500 ppm (Table 1). The least inhibition was observed at 250 ppm (11.44 mm).

Table 1. *In vitro* evaluation of antibiotics against *Xanthomonas oryzae* pv. *oryzae*

Chemicals	Inhibition zone (mm)				
	Concentration (ppm)				
	250	500	750	1000	Mean
Streptomycin sulphate	22.00	20.33	22.00	23.33	21.91
Streptocycline	20.00	22.33	24.00	25.00	22.83
Cyclohexamide	10.33	12.33	13.66	14.00	12.58
Agrimycin 100	13.66	16.00	17.33	18.00	16.25
Tetracycline	0.00	0.00	0.00	3.00	0.75
Copper oxychloride (COC)	0.00	0.00	0.00	0.00	0.00
Streptocycline + COC*	17.66	19.66	21.33	22.66	20.33
Streptocycline + Copper sulphate**	19.33	20.00	22.66	24.33	21.58
Control	0.00	0.00	0.00	0.00	0.00
Mean	11.44	12.29	13.44	14.48	12.91

Source	S.Em ±	C.D. (0.01)
Chemicals	0.157	0.590
Concentrations	0.105	0.393
Chemicals x concentration	0.315	1.179

* Both at same concentration; ** Both at same concentration

Streptomycin sulphate was significantly different among concentrations except between 500 and 750 ppm, which were on par with each other. However, 1000 ppm recorded the maximum inhibition (23.33mm) of the bacterium *X. oryzae oryzae*. Streptocycline showed the maximum inhibition at 1000 ppm. While least was recorded at 250 ppm.

Streptocycline recorded the maximum inhibition when compared to streptocycline + Copper sulphate streptocycline, streptocycline + copper oxychloride and Streptocycline + Copper sulphate (Table 1).

Field studies

Under field experiment there was no disease incidence during the first spray 30 DAT. On 90 DAT maximum per cent disease index was recorded in control (85.3) and results indicated that, all the treatments were significantly superior to control. Streptocycline + Copper oxychloride @ 0.025 + 0.1 per cent recorded the least per cent disease index of 52.7 at 90 DAT followed by Bactrinashak @ 0.04 per cent (57.9) and Bactrinashak @ 0.03 per cent (58.8) each. However, there were no significant

and there was significant difference among the differences in the per cent disease index of copper

oxychloride (0.01%) *Pseudomonas fluorescens* (1%), nimbicidin (1%), and Neem seed kernel extract + cow urine + vermiwash (5%) which were on par with each other. Among the treatments the maximum PDI was noticed in nimbicidin with 69.0 per cent (Table 2).

Similarly, streptocycline + Copper oxychloride @ 0.025 + 0.1 per cent had the highest percent reduction of disease over control (32.6 %), followed by Bactrinashak @ 0.04 per cent (27.4 %) and Bactrinashak @ 0.03 per cent (26.5%) each. While the lowest per cent reduction was observed in neem seed kernel extract + cow urine + vermiwash with 17.3 per cent. These observations were in line with the reports of Devadath and Premalatha Dath (1970); Jain, 1970; Kauffman and Kannaiyan 1987).

The data pertaining to grain yield are presented in Table 3. In the present study there was significant

Table 2. Field efficacy of antibiotics and bio-rational pesticides against bacterial blight of paddy

Treatment	Concentration (%)	PDI at 60 DAT	PDI at 90 DAT	Per cent reduction over control	Yield q/ha
Bactrinashak	0.033	71.2 * (57.593)	58.8 * (50.06)	26.5	61.82
Bactrinashak	0.041	77.7 *	57.9 * (61.82)	27.4 (49.50)	63.44
Streptocycline + Copper oxychloride	0.025 + 0.1	62.8 *	52.7 * (52.483)	32.6 (46.56)	72.02
Copper oxychloride	0.01	74.7 *	68.2 * (59.867)	17.1 (55.67)	53.10
Pseudomonas fluorescens	1	73.3 *	67.0 * (58.890)	18.3 (54.94)	55.70
Nimbicidin	1	75.3 *	69.00 * (60.290)	16.3 (56.12)	52.32
NSKE + CU + VW	5	76.2 *	68.00 * (60.843)	17.3 (55.57)	53.78
Control	-	84.6 *	85.3 * (66.873)	(67.45)	41.88
S.Em ±	-	1.949	2.29		2.404
CD at 5% P = 0.05	-	5.911	6.96		7.291

* = Original value; Values in paranthesis are angular transformed value. NSKE = Neem seed kernel extract, CU= Cow urine, VW= Vermiwash

increase in grain yield in streptocycline + copper oxychloride treatment @ 0.025 % + 0.1 with grain yield of 72.02 q/ha, which differed significantly and superior over other treatments, followed by Bactrinashak @ 0.04 per cent (63.44 q/ha) and Bactrinashak @ 0.03 per cent (61.82 q/ha). Least grain yield was obtained in nimbicidin 52.32 q/ha, while the grain yield in check plot was 41.88 q/ha.

Two sprays of Streptocycline + copper oxychloride @ 0.025 % + 0.1% not only reduced the disease incidence but also gave the higher net returns Rs.12,503.00, followed by Bactrinashak @ 0.04 per cent that gave Rs.9,778.00 and Bactrinashak @ 0.03 per cent gave Rs.9,193.67.0 respectively. This was obviously due to the higher grain yield obtained in these chemical treated plots. Least net returns were obtained in Neem seed kernel extract + Cow urine + Vermiwash (Rs.-4695.00) (Table 3).

Biochemical studies

Effectiveness of immunomodulator, biocontrol agent and botanicals on changes in total and ortho-dihydroxy phenols in leaf tissues of rice was studied (Table 4). The data indicated that the amount of total phenol and ortho-dihydroxy phenol contents

Table 3. Economics of control of Bacterial blight of paddy with antibiotics and biorational pesticides

Treatment	Concentration in %	Quantity required for two sprays (kg/ha)	Cost of chemical spray including labour charge for two sprays (Rs.)	Yield q/ ha	Yield increase over control (q/ha)	Gross return due to the chemical spray (Rs)	Net return due to the chemical spray (Rs)
Bactrinashak	0.03	0.66	1773.33	61.82	19.94	10967.00	9193.67
Bactrinashak	0.04	0.8	2080.00	63.44	21.56	11858.00	9778.00
Streptocycline + COC	0.025 + 0.1	0.5+4	4073.33	72.02	30.14	16577.00	12503.47
Copper oxychloride (COC)	0.1	4	1240.00	53.10	11.22	6171.00	4931.00
Pseudomonas fluorescens (spray)	1	20	4240.00	55.70	13.82	7601.00	3361.00
Nimbicidin	1	20 L	5200.00	52.32	10.44	5742.00	542.00
NSKE + CU + VW	5	5	11240.00	53.78	11.9	6545.00	-4695.00
Control	-	-	-	41.88	-	-	
S.Em ±				2.40			
CD@5%				7.29			

NSKE = Neem seed kernel extract, CU= Cow urine, VW= Vermiwash

increased with increased number of sprayings in all the treatments except in control.

The maximum total phenol content of 312.3 µg / g of leaf weight was noticed in *Pseudomonas fluorescens* treated leaf, followed by Bactrinashak 210.4 µg / g of leaf weight after third spray. While, the least was in water sprayed control treatment (Table 4).

Similarly, the maximum total and ortho-dihydroxy phenol content of 73.95 µg / g of leaf weight was recorded in *Pseudomonas fluorescens* treated leaf while, the least was noticed in water spray (Table 4). The results of the present investigation are in agreement with earlier studies by Sindhan and Jaglan (1988) who reported that total phenols enhanced by application of fungicides and plants

Table 4. Effect of immunomodulator, bioagents and botanical extracts on changes in total phenols and ortho-dihydroxy phenols in the leaf tissues of rice plant

Treatment	Total phenols (mg/g leaf weight)			Ortho-dihydroxy phenols		
	I	II	III	I	II	III
Bactrinashak (24 g/l)	187.5	206.3	210.4	47.50	48.64	49.23
Pseudomonas fluorescens spray (1%)	310.6	311.8	312.3	71.25	73.21	73.95
NSKE + CU + VW (5%)	170.8	173.4	174.1	50.28	51.38	52.328
Control (water spray)	130.6	128.0	126.5	30.0	30.60	29.89

NSKE = Neem seed kernel extract, CU= Cow urine, VW= Vermiwash; I = After first spray at 65 days after transplanting; II = After second spray at 75 days after transplanting III = After third spray at 85 days after transplanting

showed enhanced resistance to late leaf spot of groundnut. Even the higher levels of phenols were recorded in plants in which seeds were treated with non conventional chemicals like cycloheximides, cupric chloride, DL-phenyl alanine and indole-3-acetic acid (Chawdhury, 1995).

Bordeaux mixture, copper oxychloride, streptocycline, plantomycin and Mancozeb, copper oxychloride 0.25 + streptocycline 100 ppm were effective (Thind and Mehra, 1992) and streptocycline was recommended for seed treatment and foliar spray in India for bacterial blight management by Padmanabhan (1983); Shetty and Thirumalachar (1968).

Streptomycin is well known to interfere with ribosomes and protein biosynthesis (Traub, 1969). Although the mechanism of action of the antibiotics is highly variable, antibiotics are known to control plant diseases by acting on the parasites or on the host directly or after undergoing transformation with in the plant (Dutta, 1978). The harmful effect of streptomycin has been considered to be due to inhibition of translocation of accumulated starch and the absorption of manganese.

It has been widely recognized that one of the major biological properties of the aromatic compounds such as mono and dihydric phenols, phenolic glycosides, flavonoids, anthocyanins, aromatic amino acids and coumarin derivatives is their antimicrobial activity and it is often assumed that their main role in plants is to act as protective compounds against disease causing agents. Hence, in the present study effort has been made to find out the effect of botanical extracts, bioagents and chemical on the changes in phenolics.

Bactrinashak is an immuno modulator, when used as prophylactic treatment reduces the susceptibility of plant to bacterial diseases, by modifying the immune system of the plants. Bactrinashak alters the immune system of plant by changing the contents of phenols, protiens, nitrogen and certain enzymes and make the plants resistant to bacterial attack. Choloroplyll content is also increased (http://www.indofilcc.com).

Mohan and Mahadevan (2001a) studied the effect of phenol on protein and amino acid content of *Xanthomonas oryzae* pv. *oryzae* and reported that phenols altered the protein content and it declined with the increasing concentration of phenol. SDS-PAGE electrophoresis of protein samples of control and phenol treated cells revealed a marked difference between them. Further, the peaks of control cells, which were of large protein molecules were broken to several smaller peaks (or small peptide molecules) in the phenol treated cells. The mode of action of phenol involves substantial changes in the protein metabolism of *X. o*. pv. *oryzae*.

Fogg and Lodge (1945) proposed that the protein precipitating ability depends upon its concentration. Further, the rate of synthesis of enzymes would be reduced by the phenol, leading to decline in metabolism. The bactericidal action of phenol, according to Cooper (1912) is by precipitating proteins. Lipid profile of the cells of *X. o*. pv. *oryzae* was markedly affected by phenol treatment (Mohan and Mahadevan, 2001b)

Velusamy *et al*. (2006) reported that certain plant-associated strains of fluorescent *Pseudomonas* spp. were known to produce the antimicrobial antibiotic 2,4-diacetylphloroglucinol (DAPG). It had antibacterial, antifungal, antiviral, and antihelminthic properties and played a significant role in the biological control of tobacco, wheat and sugar beet diseases, sub population of 27 strains of plant-associated *Pseudomonas fluorescens* screened in a batch of 278 strains of fluorescent pseudomonads produced DAPG. They also report that this compound inhibited the growth of the devastating rice bacterial blight pathogen *Xanthomonas oryzae* pv. *oryzae* in

laboratory assays and suppressed rice bacterial blight up to 59%–64% in net-house and field experiments. Tn5 mutants defective in DAPG production (Phl–) of *P. fluorescens* PTB 9 were much less effective in their suppression of rice bacterial blight.

References

Anonymous, 1996. Standard evaluation system for rice. International Rice Testing programme. International Rice Research Institute Report, The Philippines.

Chawdhury, A.K. 1995. Biochemical changes associated with induction of resistance in groundnut plants to *Puccinia arachidis* by seed treatment with non-conventional chemicals. *Indian J. Mycol. Plant Pathol.,* **25**: 231- 234

Cooper, E.A. 1912. On the relations of phenol and metacresol to proteins: a contribution to our knowledge of the mechanism of disinfection. *Biochem., J*., **6**: 425

Devadath, S. and Premalatha Dath, A. 1970. Screening of antibiotics and chemicals against bacterial blight of rice. *Oryza.*, **7**: 33-38

Dutta, R.N. 1978. Effects of antibiotics on gene physiology. *M.Sc. Dissertation,* Mithila University, Darbhanga, pp. 54.

Fogg, A.H. and Lodge, R.M. 1945. The mode of antibacterial action of phenols in relation to drug fastness. *Trans Faraday Society*, **41**:359

Indofil chemicals company, web site: http:// www.indofilcc.com Mode of action of bactrinashak.

Ishiyama, S. 1922. Studies on the white leaf disease of rice plants. *Rep. Agri. Exp. Stat.,* **45**: 2361.

Islam, N. and Bora, L.C. 1998. Biological management of bacterial leaf blight of rice with plant growth promoting rhizobacteria. *Indian J. Agric. Sci.,* **68**: 798-800

Jain, S.S. 1970. Relative efficacy of some chemicals against bacterial blight of rice caused by *Xanthomonas oryzae* (Uyeda and Ishiyama) Dowson. *Oryza*, 7: 19-32

Jaypal, R. and Mahadevan, A. 1968. Biochemical changes in banana leaves in response of leaf spot pathogenesis. *Indian Phytopathol.*, **21**: 43-48

Kauffman, .E. and Kannaiyan, S. 1987. Bacterial blight of rice. In: *Advances in rice Pathology.* Ed. Kannal yan, S, Tamil Nadu Agricultural University, Coimbatore, p. 265-272.

Kumar, S.M., Singh, H.S. and Sharma, D. 1999. Survey of disease and pathogenic reaction of *Xanthomonas oryzae* pv. *oryzae* in Balaghat region of Madhya Pradesh, India. *Ann. Pl. Protec. Sci.,* **7**: 118-119

Mohan, N. and Mahadevan, A. 2001a. Effect of phenol on protein and amino acid content of *Xanthomonas oryzae* pv. *oryzae , Indian J. Exp. Bio*., **39**: 1055-1061

Mohan, N. and Mahadevan, A. 2001b. Effect of phenol on lipid and fatty acid profile of *Xanthomonas oryzae* pv. *oryzae ,Indian J. Exp. Bio*., **39**: 1062-1064

Ou, S.H. 1985. *Rice Diseases,* second Edition, Common Wealth Mycological Institute, Kew, England. pp. 370.

Padmanabhan, S.Y. 1983. Integrated control of bacterial blight of rice. *Oryza.,* **20**: 188-193

Shetty, K.S. and Rangaswamy, G. 1971. *In vitro* and *in vivo* activities of streptocycline on bacterial blight of rice caused by *Xanthomonas oryzae*. *Indian Phytopathol.,* **24**: 145-152

Sindhan, G.S. and Jaglan, B.S. 1988. Effect of fungicides on the biochemical response of groundnut plant against tikka leaf spot. *Pl. Dis. Res.,* **31**: 57-59

Singh, R.S. 2002. *Introduction to Principles of Plant Pathology,* 4th Edition, Oxford and IBH Publishing Co. Pvt. Ltd., New Delhi, p. 290.

Swings, J., Van Den Mooter, M., Vauterin, L., Hostee, B., Gillis, M., Mew, T.W. and Kerster, K. 1990. Reclassification of the causal agents of bacterial blight (*Xanthomonas campestris* pv. *oryzae*) and bacterial leaf streak (*Xanthomonas campestris* pv. *oryzicola*) of rice as pathovars of *Xanthomonas oryzae*(Ishiyama) *Rev*. *Intern. J. Syst. Bacteriol.,* **40**: 309-311

Thind, B.S. and Mehra, R.K. 1992. Chemical control of bacterial blight of rice. *Pl. Dis. Res.,* **7**: 226-234

Thirumalachar, M.J. 1968. Antibiotics in the control of plant pathogens. *Adv. Appl. Microbiol.,* **10**: 313-337

Traub, P. 1969. *Inhibitors: tools in cell research.* Ed. Buchler and H. Sies, Springer Verlag, Berlin, pp. 79-99.

Velusamy, P., Immanuel, J.E., Gnanamanickam, S.S. and Thomashow, L.S. 2006. Biological control of rice bacterial blight by plant-associated bacteria producing 2,4- diacetylphloroglucinol. *Canadian J. Microbiol*., **52**: 56-65.

Received: March 14, 2012; Accepted: May 14, 2012