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Balanagouda Patil
Department of Plant Pathology, College of Agriculture, University of Agricultural and Horticultural Science, Shivamogga, Karnataka, India

Jagadeesh GB
Extension Education Unit, Agricultural and Horticultural Research Station, Kathalagere, Davanagere, Karnataka, India

Karegowda C
Department of Plant Pathology, College of Agriculture, University of Agricultural and Horticultural Science, Shivamogga, Karnataka, India

Revathi RM
Department of Plant Pathology, College of Agriculture, University of Agricultural and Horticultural Science, Shivamogga, Karnataka, India

Seema Naik
Department of Plant Pathology, College of Agriculture, University of Agricultural and Horticultural Science, Shivamogga, Karnataka, India

Correspondence
Balanagouda Patil
Department of Plant Pathology, College of Agriculture, University of Agricultural and Horticultural Science, Shivamogga, Karnataka, India

Efficacy of botanicals, bio agents and antibacterial chemicals against *Xanthomonas oryzae* pv. *oryzae* under *in vitro* condition

Balanagouda Patil, Jagadeesh GB, Karegowda C, Revathi RM and Seema Naik

Abstract
Bacterial leaf blight of rice caused by *Xanthomonas oryzae* pv. *oryzae* is menace in rice cultivation. Some of the chemicals are effective against disease but they have not proved economical and they leave harmful residues in soil and plants. Therefore, present investigation was taken up to manage the disease with help of botanicals, bio agents and chemicals which have bactericidal action. Hence, twelve botanicals, three bio agents and nine antibacterial chemicals were evaluated against pathogen and results revealed that among botanicals tested, maximum inhibition zone of 15.00 mm was recorded by marigold which was superior over all other botanicals and concentrations tested followed by neem (14.00 mm). Whereas, least inhibition zone of 7.00 mm was recorded by patchouli and tridax extracts. While *Trichoderma harzianum* was found significantly superior in inhibiting the growth of pathogen (22.86 mm) followed by *Pseudomonas fluorescens* (17.20 mm). Among the chemicals kasugamycin was significantly superior over all the chemicals with mean inhibition zone of 16.60 mm followed by bacterinashak, streptocycline + copper oxychloride with mean inhibition zone of 16.50 mm and 14.10 mm respectively.

Keywords: *Xanthomonas oryzae* pv. *oryzae*, bio agents, botanicals, antibacterial chemicals

Introduction
Rice (*Oryza sativa* L.) is one of the most widely cultivated food crops of the world and its production is reducing by many diseases caused by fungi, bacteria and viruses. Among all the disease infecting rice, bacterial leaf blight (BLB) or bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae* [1] is one of the most destructive diseases of rice in both irrigated and rainfed ecosystem [2]. It is considered to be a major constraint for low rice productivity iii tropical Asia. It was first reported from India by [3] from Maharashtra State. It is typical vascular disease, systemic in nature. The disease causes infection at nursery seedling, after transplanting and later at booting or heading stage. Its Kresek phase is most serious and destructive. Some of the chemicals are effective against this disease but they have not proved economical and leave harmful residues in soil and plants. In recent years emphasis has been given on eco-friendly management practices. Therefore present investigation was planned to manage the disease with the help of botanicals, bio agents and some chemicals which have bactericidal action. The effort has been made to evaluate the efficacy of botanicals, bio agents and antibacterial chemicals against the pathogen under laboratory condition.

Materials and Methods
Efficacy of Botanicals
Twelve botanicals *viz.,* Neem (*Azadirachta indica*), Glyricidia (*Glyricidia Spp*), Honge (*Pongamia pinnata*), Marigold (*Tagetes Spp*), Lantana (*Lantana camara*), Nilgiri (*Eucalyptus Spp*), Noni (*Morinda citrifolia*), Mehendi (*Lawsonia inermis*), Patchouli (*Pogostemon cablin*), Garlic (*Allium sativum*), Onion (*Allium cepa*) and Tridax (*Tridox procumbens*) were tested for their bactericidal properties against *Xanthomonas oryzae* pv. *oryzae*. Plant extracts were prepared from freshly collected plant parts and these were assessed @ 2.5%, 5%, 10 % concentration by paper disc method. A heavy suspension of the *X. oryzae* pv. *oryzae* was prepared and poured into sterile Petri plates and allowed to solidify.

Sterilized filter paper disc measuring 5 mm in diameter was soaked in different concentrations of the plant extracts, were dried and placed on solidified medium. The plates were then incubated at 28 ºC for 48 to 72 hrs. Observations on inhibition zone were recorded after the incubation period with three replications.

Efficacy of bio agents

To determine the inhibitory effect of certain bio agent's *viz.*, *Pseudomonas fluorescens, Bacillus subtilis* and *Trichoderma harzianum* were evaluated under *in vitro* condition. Antagonistic activity of bio agents were judged against promising isolate of *Xanthomonas oryzae* pv. *oryzae* by paper disc method. In this method culture filtrate were poured in 5mm diameter paper formed in each petriplates containing nutrient agar seeded with pathogen. The plates were kept incubation @ 28 ºC for 48 to 72 hrs. The potential antibacterial property was calculated by comparing zone of inhibition with that of control after 3 days incubation.

Efficacy of antibacterial chemicals

Toxicity of chemicals @ 100ppm, 500ppm, 1000ppm concentration against *Xanthomonas oryzae* pv. *oryzae* were studied by paper disc method. Antibacterial chemicals used for the study are Bacterinashak, Copper oxychloride + streptocycline, Copper oxychloride, Kasugamycin, Streptocycline, Streptomycin sulphate, Copper hydroxide, Nimbicidine and Agrimycin 100. In this method, sterilized whatman filter paper (5mm) was used for study. These discs were impregnated with requisite quantity of chemicals for 30 minutes. Impregnated paper discs were aseptically placed on nutrient agar plates, containing 24 hours old bacterial culture. The discs moistened with sterilized distilled water served as control. All plates were kept incubation @ 28ºC for 48 to 72 hrs. After incubation period observations were recorded based on zone of inhibition. Collected data were analyzed statistically.

Results and Discussion

Efficacy of botanicals

Efficacy of 12 botanicals @ 2.5, 5 and 10% concentration were tested against *Xanthomonas oryzae* pv. *oryzae*. The data regarding the per cent inhibition over control were presented in Table1. Among the twelve botanicals tested, maximum inhibition zone of 15.00 mm was recorded by Marigold leaf extract at 10 per cent concentration which was superior over all other botanicals and concentrations tested, followed by Neem leaf extract (14.00 mm). Whereas, least inhibition zone of 7.00 mm was recorded by Patchouli and Tridax leaf extracts at 2.5 per cent concentration.

Marigold leaves contain flavonoids and tannins which inhibit bacterial growth. Neem leaf extracts contains quercetin (flavonoid) and nimbosterol (ß- sitosterol) as well as number of liminoids (nimbin and its derivatives). Quercetin (a polyphenolic flavonoid) is known to have antibacterial and antifungal properties. Results were in line with the findings of [4] reported that of the seven commercial formulations of botanicals, neem gold was found best which gave maximum inhibition zone (13.6 mm) at 2000 ppm followed by neemazal (10.6mm). Among five plant extracts, neem leaf extract was found best. Present finding similar with the findings of [5, 7]. They also reported the inhibitory effect of *Azadirachta indica*, *Curcuma longa*, *Datura metal* and *Zingiber officinale* against *Xantlwmonas oryzae* pv. *oryzae.*

Efficacy of bio agents

Antagonistic effect of different bio agents on the growth of *Xanthomonas oryzae* pv. *oryzae* was studied under *in vitro* condition. Results revealed from Table 2, among the three bio agents tested *Trichoderma harzianum* was found significantly superior in inhibiting the growth of *X. oryzae* pv. *oryzae* (22.86 mm) followed by *Pseudomonas fluorescence* (17.20 mm). However, the least inhibition zone of 15.00 mm was observed in *Bacillus subtilis* against the growth of pathogen.

These findings were supported by [8] who evaluated *P. fluorescens in vitro* by dual culture method, revealed that it produced inhibition zones of 4.24 mm against *X. oryzae* pv. *oryzae*, whereas evaluation by culture filtrate method produced inhibition zones of 14.75mm. Similar results were obtained by [9] among the bio-agents tested *in vitro*, *P. fluorescens* was found little bit effective with an inhibition zone of 3.8 mm at 5000 ppm (0.5%) concentration and *T. viridae* exhibited as no growth of *X. oryzae* pv. *oryzae*.

Efficacy of antibacterial chemicals

Sensitivity of antibacterial chemicals against pathogen was conducted through paper disc or inhibition zone method. Data pertaining to inhibition zone (mm) are depicted in Table 3.

Differences among treatments and concentrations were found to be statistically significant. Among nine chemicals tested, kasugamycin was significantly superior over all the chemicals with mean inhibition zone of 16.60 mm followed by bacterinashak, streptocycline+ Copper oxychloride with mean inhibition zone of 16.50 mm and 14.10 mm respectively. Whereas, least mean inhibition zone of 11.10 mm was recorded by agrimycin 100.

This findings were in accordance with [10] reported that streptocycline (streptomycin sulphate + tetracycline hydrochloride, 9:1) + copper sulphate ($CuSo_4$) at 1000 ppm showed the maximum inhibition zone of 25.0 mm, followed by streptomycin sulphate + copper oxychloride (24.33mm), streptomycin sulphate alone (22.66mm) and agrimycin 100 (16.25mm). Similar results were obtained by [11] reported that, copper hydroxide2.5% was very effective in controlling the growth of the *X. oryzae* pv. *oryzae* with 6.4 per cent inhibition and followed by validamycin at 2.5 per cent with 5.56 per cent inhibition over control.

Table 1: *In vitro* evaluation of botanicals against *Xanthomonas oryzae* pv. *Oryzae*

S. No	Botanicals	Mean diameter of inhibition zone (mm)			Mean
		Concentration (%)			
		2.5	5	10	
1.	*Glyricidiasepium*	9.0(3.239)*	10.0 (3.380)	14.0 (4.067)	11.00
2.	*Azadirachtaindica*	10.0 (3.380)	11.0 (3.531)	14.0 (4.067)	11.60
3.	*Eucalyptus globulus*	7.1 (3.079)	10.0 (3.380)	13.0 (3.936)	10.00
4.	*Aliumsativum*	7.2(3.083)	8.0 (3.136)	10.0 (3.380)	8.30
5.	*Lanatanacamara*	10.0 (3.380)	11.0 (3.531)	11.0 (3.239)	10.60
6.	*Allium cepa*	10.0 (3.380)	11.0 (3.531)	12.0 (3.603)	11.00

7.	*Pongamiapinnata*	9.0 (3.239)	10.0 (3.380)	9.0(3.239)	9.30
8.	*Tridaxprocumbens*	7.0 (3.073)	7.0 (3.073)	9.0 (3.239)	7.60
9	*Morindacitrifolia*	12.0 (3.603)	13.0 (3.936)	13.0 (3.936)	12.60
10	*Pogostemoncablin*	7.0 (3.073)	11.0 (3.531)	13.0 (3.936)	10.30
11	*Tagetes* spp.	9.0 (3.239)	12.0 (3.603)	15.0 (4.165)	12.00
12	*Lawsoniainermis*	9.0 (3.239)	11.0 (3.531)	12.0 (3.603)	10.60
13	Control	0.0 (0.701)	0.0 (0.701)	0.0 (0.701)	0.00
	Mean	8.10	10.30	11.20	-
	Factors	Botanicals(B)	Concentration(C)	BxC	-
	SE.m±	0.05	0.02	0.09	-
	CD at 1%	0.17	0.08	0.29	-

*Figures in the parenthesis are square toot transformed values

Table 3: *In vitro* evaluation of antibacterial chemicals against *Xanthomonas oryzae* pv. *Oryzae*

S. No	Chemicals	Mean diameter of inhibition zone (mm)			Mean
		Concentration (ppm)			
		100	500	1000	
1.	Copper Oxy Chloride	10.0 (3.875)*	12.0 (4.181)	15.0 (4.301)	12.3
2.	Copper Hydroxide	11.0 (3.74)	12.0 (3.803)	13.5 (3.936)	12.1
3.	Kasugamycin	14.0 (3.239)	16.0 (3.536)	20.0 (3.74)	16.6
4.	Streptocycline	11.0 (3.38)	12.0 (3.536)	15.0 (3.936)	12.6
5.	Streptocycline + Copper Oxy Chloride	13.5 (3.803)	14.0 (4.067)	15.0 (4.527)	14.1
6.	Streptomycin sulphate	11.0 (3.380)	14.0 (3.536)	16.0 (3.936)	13.6
7.	Agrimycin 100	9.0 (3.936)	11.0 (3.380)	13.5 (4.067)	11.1
8.	Nimbicidine	10.0 (3.239)	13.0 (3.675)	16.5 (4.129)	13.1
9.	Bacterinashak	14.5 (3.073)	17.0 (3.380)	18.0 (3.740)	16.5
10.	Control	0 (0.701)	0 (0.701)	0 (0.701)	0.00
	Mean	10.4	12.1	14.2	-
		Chemicals(C)	Concentration(C)	CxC	-
	SEm±	0.03	0.01	0.06	-
	CD at 1%	0.12	0.06	0.21	-

*Figures in the parenthesis are square root transformed values

Table 4: *In vitro* evaluation of bio-agents against *Xanthomonas oryzae* pv. *Oryzae*

S. No	Name of the bio-agents	Mean diameter of inhibition zone (mm)
1.	*Pseudomonas fluorescense*	17.20 (4.14)*
2.	*Bacillus subtilis*	15.00(3.87)
3.	*Trichoderma harzianum*	22.86 (4.78)
	SEm±	0.56
	CD at 1%	1.77

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