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RESEARCH ARTICLE

In vitro antibacterial activity of plant extracts against *Xanthomonas oryzae* pv. *oryzae* causing bacterial leaf blight in rice

■ L. VENGADESH KUMAR* AND P. BALABASKAR

Department of Plant Pathology, Faculty of Agriculture, Annamalai University, Annamalai Nagar, CHIDAMBARAM (T. N.) INDIA

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*Corresponding author:
kumarvengadesh@yahoo.co.in

ABSTRACT

In the present study, an attempt has been made to use plant extracts in place of synthetic chemicals to reduce *Xanthomonas oryzae* pv. *oryzae* incidence. Accordingly, the antibacterial activity of 40 plant extracts was assessed and out of which ten plant extracts were found to have appreciable antibacterial activity against *Xanthomonas oryzae*. Among the plant extracts, *Rhizophora apiculata* at 20 per cent showed a large inhibition zone with high activity index and recorded maximum plant vigor index. This was followed by *Aadathoda vesica* and *Punica granatum* at 20 per cent concentration. The efficacy of these ten plant extracts was also tested through detached leaf assay on rice variety ADT 43. The protective treatment (before inoculation) was found to be significantly more effective than the curative treatment (after inoculation) and the most effective plant extract was found to be *R. apiculata*. Rice plants treated with *R. apiculata* developed the smallest lesion which was followed by *Aadathoda vesica*, *Punica granatum*, *Tamarinda indicus* and *Datura stramonium* in the decreasing order of merit. Under pot culture conditions also, the extract of *R. apiculata* showed maximum efficacy against bacterial leaf blight incidence recording minimal lesion size when compared to other treatments.

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INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most widely cultivated food crops and one of the major cereals used all over the world (Salim *et al.,* 2003). Tropical and sub-tropical regions of the world are the major rice-producers, with 90 per cent of production occurring in Asia (Ezuka and Kaku, 2000). Among the various pests and diseases the bacterial leaf blight (BLB) of rice caused by *Xanthomonas oryzae* pv. *oryzae* is the most devastating and harmful limiting factor in rice production especially in South East Asia, since the widespread cultivation of dwarf high-yielding cultivars. In India, yield loss up to 81.3 per cent (Gnanamanickam *et al*., 1999; Veena *et al*., 2000) has been reported due to this disease and in Japan the yield losses ranging between 20-30 per cent and occasionally up to 50 per cent have been reported (Ou, 1985).

X. oryzae pv. *oryzae* is known to be seed-borne and seed transmitted (Veena *et al*., 2000). The contaminated seeds results in poor germination and severe infection and affect the plants at tillering and flowering stages, thereby resulting in the formation of chaffy seeds. In addition to direct yield loss, this disease also adversely affects the seed quality through seed discoloration.

Unsuccessful attempts have been made to manage this disease using chemotherapeutics that are expensive and affect the beneficial microorganisms which prompted us to develop alternative management strategies. Further, excessive chemical use had a detrimental effect on consumers' health, harmful to

beneficial predators, parasitoids and the environment (Keifer and Mahurin 1997; Susan *et al.,* 2001).

Certain plants have been known for their medicinal and antimicrobial properties since ancient times. Biologically active compounds that effectively control various pests and pathogens are known from approximately 2400 plant species (Saleem, 1988). Plant origin 'biocides' are non-phytotoxic, relatively safe, systemic and easily biodegradable (Kagale *et al.,* 2004). The antibacterial activities of different plant extracts against plants diseases have been previously investigated (Aktar *et al*., 1997; Leksomboon *et al.,* 2001; Okigbo and Nmeka, 2005; Govindappa *et al*., 2011; Rukshana *et al*., 2012).

Alkaloids occurring as common salts in plants are the most important active chemicals found in plant extracts. Many secondary metabolites demonstrate high efficacy against microbes. Saponified and un-saponified fractions of brown and green seaweed were found to be very effective against *Xanthomonas oryzae* pv. *oryzae* (Arun Kumar and Rengasamy, 2000). Therefore, in the present study, an attempt has been made to evaluate the plant extracts in place of synthetic chemicals not only to reduce *X. oryzae* pv. *oryzae* incidence but also to assess its effect on crop growth and yield of paddy.

MATERIALS AND METHODS

Pathogen collection :

Leaves showing typical bacterial blight symptoms were selected and cut into small bits of 3 mm in size. The leaf bits were surface sterilized with 1 per cent sodium hypochlorite solution and washed through a series of Petri plates containing sterile distilled water. These leaf bits were again submerged in test tubes containing 3 ml of sterile water and incubated for 6 h at room temperature. Such inoculated water was streaked with the help of sterile inoculation needle over the Petri plates containing Peptone sucrose agar medium.

After 48 h of incubation at room temperature (28±2^{0}C), the yellowish bacterial growth on the medium was subcultured in slants and purified by the dilution plate technique. Pure culture of the pathogen was maintained in nutrient agar slants at 10^{0}C.

Sample collection :

Plant samples were collected from SKM Pvt. Ltd., Erode, TN or purchased from the local market.

Plant extracts preparation :

Fresh leaves of plants were harvested and thoroughly washed in tap water 3 to 4 times. 100 g of leaves each were macerated to paste with the help of sterilized mortar and pestle with 100 ml of sterilized distilled water and it was filtered through the muslin cloth. The extract was then centrifuged at 5000 rpm for 15 min. (Govindappa *et al*., 2011). The aseptically filtered extract formed the standard plant extract solution and was stored in refrigerator for further use. This standard extract was further diluted to the required concentration using sterile distilled water. All the extracts were used at 100 per cent concentration for screening antibacterial activity. The plant species showing effectiveness in the preliminary screening were further diluted to different concentrations (5%, 10%, 15% and 20%) and tested against *Xanthomonas oryzae* pv. *oryzae* under *in vitro*.

Antibacterial activity of plant extracts against *Xanthomonas oryzae* pv. *oryzae* :

The potency and activity of antimicrobials are usually determined by zone of inhibition they produce when they act upon bacteria grown on agar plates. Agar disc diffusion methods are the most frequently applied assay methods. Sterile nutrient broth was prepared and inoculated with the test organisms under aseptic conditions. It was incubated for 24 h at 37^{0}C and used as inoculum. Bacterial suspension of *Xoo* was prepared in sterile distilled water to a concentration of 10^8 colony forming units (CFU)/ml. One ml of bacterial cell suspension was mixed with 19 ml of Nutrient agar (NA) medium and poured onto the sterile Petri dishes. After solidification, sterile paper discs (6 mm diameter) were placed on the surface of the medium at 1 cm away from the periphery of Petri dish and 50µl of the supernatant fractions of plant extract were applied to each disc. Standard streptomycin (100 ppm) discs were used as positive control while distilled water (50µL/disc) served as negative control. The experiment was performed in triplicates under aseptic condition. The plates were incubated at room temperature for 48 h and the antibacterial activity was evaluated by measuring the inhibition zones (including diameter of the disc). The mean value of the diameter of the inhibition zone of the triplicates was taken as the final value.

Plant growth promotion (*in vitro*) Roll towel method :

Plant growth-promoting activity of the plant extracts was assessed based on the seedling vigour index by the standard roll towel method (ISTA, 1993). Twenty five rice (ADT 43) seeds treated with plant extracts were kept over the pre-soaked germination paper. The seeds were held in position by placing another pre-soaked germination paper strip over it and gently pressed. The sheets along with seeds were then rolled and incubated in growth chamber for 10 days. Three replications were maintained for each treatment. The root length and shoot length of individual seedlings were measured and the per cent germination of seeds was also calculated. The seedling vigour index was calculated.

Vigor index (VI) = (Mean root length + Mean shoot length) × Germination (%)

Detached leaf assay :

Protective method :

The young leaves of rice variety ADT-43 were dipped in different concentrations (5, 10, 15 and 20%) of plant extract for 5-10 min and inoculated with *Xoo* suspension (10^8 cfu/ml) using the pin prick method. Three leaves were kept on three layers of water-saturated blotting paper in Petri plates. The leaves dipped in Streptomycin (100 ppm) and sterile distilled water and then inoculated with the most aggressive isolate served as comparison and the leaves dipped in sterile distilled water and inoculated with the pathogen served as control. Three plates of each treatment and a control were incubated at 28±2°C for 48 h. After incubation the lesion length was measured in cm.

Curative method :

The leaves of rice variety ADT -43 were inoculated with a suspension (10^8cfu/ml) of the *Xoo* following pin prick method. Plant extract of different concentrations (5, 10, 15 and 20%) were applied on leaves placed in glass Petri plates. The control leaves were dipped in sterile distilled water. Standard Streptomycin (100 ppm) treated leaves were used for comparison. The plates were sealed with adhesive tape and incubated at 28±2°C for 48 h.

Pot culture assay :

The rice plants were inoculated by clipping method (Kauffman *et al*., 1973) at boot leaf stage with a pair of scissors every time dipped into the bacterial suspension containing10^8cfu/ml, prepared from 48 h old actively growing culture grown on nutrient agar medium. After two days of inoculation plant extract were sprayed with hand sprayer and covered with polythene bags. The control plants were treated with distilled water. In protective method, the plant extracts were applied before inoculation and in curative method, the plant extracts were applied after inoculation of test bacterium. After 24 h, the bags were removed and the lesion length was measured in cm after 21 days.

RESULTS AND DISCUSSION

The antibacterial activity of 40 plant extracts (100g/100 ml) against *Xanthomonas oryzae* were assessed (Table 1). Only ten plant extracts (*Aadathoda vesica, Calotrophis jaijantia, Cinnamom zylanicum, Curcuma longo, Datura stramonicum, Eugenia caryophyllata, Lawsonia inermis, Punica granatum, Rhizophora apiculata* and *Tamarinda indicus*) were found to have appreciable antibacterial activity. The best results were shown by *Rhizophora apiculata,* which showed a large inhibition zone (15.43mm) with a high activity index value (0.85). This was followed by *Aadathoda vesica* (14.32 mm) and *Punica granatum* (13.10 mm). The least result was recorded by *Eugenia caryophyllata* (5.72 mm). Streptomycin (100 ppm) used for comparison recorded 18 mm inhibition zone with an activity index of 1.0. (Table 1).

Out of the plant extracts tested, selectively 10 plant extracts which showed promising results were further evaluated at different concentrations (5, 10, 15 and 20%) against *Xoo*. The inhibition of growth of bacterium increased with an increase in concentration of the aqueous extract of the test plants. Extract of *R. apiculata* (15.13 mm) showed maximum inhibition with high activity index (0.84) at 20% concentration. This was followed by *Aadathoda vesica* (14.02 mm) *Punica granatum* (12.92 mm) with the activity index of 0.7 at 20 per cent concentration. The least effect was recorded by *Eugenia caryophyllata* (5.42 mm). Streptomycin (100 ppm) used for comparison recorded 18 mm inhibition zone with the activity index of 1.0 (Table 2).

Plant growth promotion :

Out of the selected plant extracts *R. apiculata* recorded the highest shoot length (14.64 cm), root length (23.51 cm) and plant vigor index (3738.70) which was significantly superior over other extracts and untreated control. This was followed by the extracts of *A. vesica* and *P. granatum* in the decreasing order of merit. The least vigour index was recorded by *E. caryophyllata* (2390.0) (Table 3).

Protective and curative effect of plant extracts :

The efficacy of ten plant extracts was also tested through detached leaf assay on rice variety ADT 43 against *Xanthmonas oryzae* isolate *Xoo* 21. The protective treatment (before inoculation) was found to be significantly more effective than the curative treatment (after inoculation) and the most effective plant extract was found to be *R. apiculata.* Rice plants treated with *R. apiculata* developed the smallest lesions (3.11 cm) which was followed by *A. vesica* (3.20 cm), *P. granatum* (3.45 cm), *T. indicus* (3.54 cm) and *D. stramonium* (4.20cm) in the decreasing order of merit. The treatment with *E. caryophyllata* recorded longer lesions (8.80cm) (Table 4).

The same ten plant extracts were also tested under pot culture conditions with rice variety ADT 43 for the management of BLB of rice. The extract of *R. apiculata* showed maximum efficacy against bacterial leaf blight in the pot culture assay, with small lesions for protective (3.10 cm) and for curative (4.0 cm) treatments. Various plant extracts have been reported to possess differing level of antimicrobial activities under *in vitro* conditions against phytopathogenic bacteria. In the present study, the extracts of *R. apiculata, A. vesica* and *P. granatum* showed significant inhibitory action against *Xoo*. The potential of plant extracts for controlling citrus canker, tested through glass house assays, has been reported by Leksomboon *et al*. (2001), who found that *Tamarindus indica* extract effectively inhibited citrus canker disease in lime.

Table 1: Antibacterial activity of plant extracts against *X. oryzae* pv. *oryzae*

Sr.No.	Plant extracts (100% conc.)	Vernacular name	Parts used	Inhibition zone (mm)	Activity index
1.	*Aadathoda vesica*	Adathodai	Leaves	14.32	0.79
2.	*Acalipha indica*	Kuppaimeni	Leaves	0.0	0.0
3.	*Acorus calamus*	Vasambu	Leaves	0.0	0.0
4.	*Allium cepae*	Vengayam	Bulb	0.0	0.0
5.	*Allium sativam*	Poondu	Bulb	0.0	0.0
6.	*Aloe vera*	Katralai	Leaves	0.0	0.0
7.	*Andrographis paniculata*	Nila vembu	Leaves	0.0	0.0
8.	*Aristalochia breateata*	Aadutheenda paalai	Leaves	0.0	0.0
9.	*Azadiracta indica*	Vembu	Leaves	0.0	0.0
10.	*Brassica campestris*	Mustard	Seed	0.0	0.0
11.	*Calotrophis jaijantia*	Erukan	Leaves	6.61	0.36
12.	*Capsicum annum*	Chilli	Leave	0.0	0.0
13.	*Catharanthus roseus*	Catharanthus	Leaves	0.0	0.0
14.	*Cinnamom zylanicum*	Elavangapattai	Bark	8.00	0.44
15.	*Cissus quadrangularis*	Perandai	Leaves	0.0	0.0
16.	*Coleus aromaticus*	Oama valli	Leaves	0.0	0.0
17.	*Curcuma longo*	Manjal	Rhizome	9.41	0.52
18.	*Datura stramonicum*	Umathai	Seeds	10.84	0.60
19.	*Eucalyptus globules*	Eucalyptus	Leaves	0.0	0.0
20.	*Eugenia caryophyllata*	Kirambu	Bud	5.72	0.32
21.	*Jatropha curcas*	kaattamanaku	Leaves	0.0	0.0
22.	*Lantana camera*	Road side weed	Leaves	0.0	0.0
23.	*Lawsonia inermis*	Henna	Leaves	9.21	0.51
24.	*Leucas aspera*	Thumbai	Leaves	0.0	0.0
25.	*Moringa oleifera*	Murungai	Leaves	0.0	0.0
26.	*Nigella sativa*	Karum seeragam	Seeds	0.0	0.0
27.	*Ocimum sanctum*	Tulsi	Leaves	2.50	0.13
28.	*Pavonia zeylanica*	Palaver	Root	0.0	0.0
29.	*Phyllanthus indica*	Nelli	Leaves	0.0	0.0
30.	*Prosopis juliflora*	Karuvai	Leaves	2.81	0.15
31.	*Psidium guajava*	Koyya	Leaves	0.0	0.0
32.	*Punica granatum*	Mathulai	Leaves	13.10	0.72
33.	*Rhizophora apiculata*	Surapunnai	leaves	15.43	0.85
34.	*Rhizophora mucronata*	Surapunnai	leaves	0.0	0.0
35.	*Ricinus communis*	Aamanakku	leaves	0.0	0.0
36.	*Spharanthus indicus*	Kottai karanthai	Seeds	0.0	0.0
37.	*Tamarinda indicus*	Tamarind	Fruit	12.00	066
38.	*Vernnonia anthelmintica*	Kattuseeragam	Seeds	0.0	0.0
39.	*Vitex negundo*	Notchi	Leaves	0.0	0.0
40.	*Zingiber officinale*	Ginger	Rhizome	1.80	0.1
41.	Streptomycin(100ppm)			18.00	1.0
42.	Control			0.0	0.0
43.	S.E.±			0.01	-
44.	C.D.(P=0.05)			0.02	-

Also, in the present study it was observed that the protective treatment (before inoculation) was found to be significantly more effective. This finding was supported by the results of Kagale *et al*. (2004) who reported antibacterial activity and induction of systemic resistance by extracts of *Datura metal* and found that extracts of *Datura metel* effectively reduced the incidence of bacterial leaf blight of rice under glass house conditions when a foliar treatment was applied before inoculation.

Similarly, the aqueous leaf extract of *A. vasica* was found to be highly effective in inhibiting the growth of *Xoo* compared to the leaf extract of *L. camera* and bulb extract of *A. sativum* (Govindappa *et al*., 2011). A decoction of *Curcuma longa* demonstrated the highest effectiveness in terms of regulating BLB in the rice plants both under laboratory and field conditions (Rukhsana, 2011). Likewise, moderate inhibitory action against *Xoo* by the extract of *Andrographis serpyllifolia* (leaves) was also reported by Jamuna Bai *et al*. (2011).These earlier reports are in line and lend support to the present work.

Table 2: Effect of selected plant extracts on the growth of *X. oryzae* pv. *oryzae* (paper disc assay)

Sr. No.	Plant extracts	Inhibition zone (mm)				Activity index			
		5%	10%	15%	20%	5%	10%	15%	20%
1.	*Aadathoda vesica*	11.00	11.88	13.01	14.02	0.61	0.66	0.72	0.77
2.	*Calotrophis jaijantia*	3.90	4.01	5.11	6.30	0.21	0.22	0.28	0.35
3.	*Cinnamom zylanicum*	5.11	5.73	7.00	7.60	0.28	0.31	0.38	0.42
4.	*Curcuma longo*	6.21	6.90	8.00	9.11	0.34	0.38	0.44	0.50
5.	*Datura stramonicum*	7.13	8.00	9.63	10.44	0.39	0.44	0.53	0.58
6.	*Eugenia caryophyllata*	3.00	3.66	4.33	5.42	0.16	0.20	0.24	0.30
7.	*Lawsonia inermis*	6.00	6.80	8.00	8.82	0.33	0.37	0.44	0.49
8.	*Punica granatum*	10.00	10.84	12.00	12.92	0.55	0.60	0.66	0.71
9.	*Rhizophora apiculata*	12.50	12.82	14.00	15.13	0.69	0.71	0.77	0.84
10.	*Tamarinda indicus*	9.22	10.00	10.92	11.71	0.51	0.55	0.60	0.65
	Streptomycin (100 ppm)	18.00				1.0			
	Control	0.0				0.0			
	S.E.±	0.02	0.08	0.02	0.01	-			
	C.D. (P=0.05)	0.08	0.19	0.05	0.03	-			

Table 3 : Plant growth promoting activity of selected plant extracts on rice cultivar (ADT 43) (Roll towel method)

Sr. No.	Plant extracts	Root length (cm)	Shoot length (cm)	Germination (%)	Plant vigour index
1.	*Aadathoda vesica*	22.43	14.30	96	3526.08
2.	*Calotrophis jaijantia*	15.61	13.00	88	2517.68
3.	*Cinnamom zylanicum*	16.50	13.20	90	2673.00
4.	*Curcuma longo*	18.53	13.50	92	2946.76
5.	*Datura stramonicum*	19.55	13.52	93	3075.51
6.	*Eugenia caryophyllata*	14.63	12.53	88	2390.08
7.	*Lawsonia inermis*	17.52	13.42	90	2784.60
8.	*Punica granatum*	21.43	14.11	95	3376.30
9.	*Rhizophora apiculata*	23.51	14.64	98	3738.70
10.	*Tamarinda indicus*	20.63	13.82	94	3238.30
	Control	18.0	6.0	80	1920.00
	S.E.±	0.01	0.01	0.05	
	C.D. (P=0.05)	0.02	0.03	0.13	

Table 4 : Protective and curative effects of selected plant extract at different conc. against BLB

Sr. No.	Treatments	Method	Detached leaf assay				Pot culture assay			
			Bacterial lesion length(cm)/ isolates							
			5%	10%	15%	20%	5%	10%	15%	20%
1.	*Aadathoda vesica*	Curative	19.63	12.71	7.43	4.23	19.81	12.38	9.84	4.20
		Protective	19.00	11.81	6.71	3.20	18.51	11.72	9.43	3.31
2.	*Calotrophis jaijantia*	Curative	22.80	13.83	11.51	7.43	19.42	18.32	12.70	8.43
		Protective	21.43	13.24	9.23	5.61	17.40	16.81	10.40	7.30
3.	*Cinnamom zylanicum*	Curative	21.73	12.64	10.55	7.64	20.82	17.44	12.70	7.81
		Protective	20.84	12.00	8.81	4.80	20.00	15.54	11.20	5.40
4.	*Curcuma longo*	Curative	21.40	17.36	8.81	5.50	20.43	14.82	12.11	6.44
		Protective	20.72	15.51	8.24	4.41	20.22	14.16	10.73	4.32
5.	*Datura stramonicum*	Curative	21.72	14.30	8.32	5.51	20.04	13.81	11.63	4.83
		Protective	21.72	13.32	8.00	4.20	18.74	12.43	10.21	3.74
6.	*Eugenia caryophyllata*	Curative	21.82	18.63	15.30	12.43	20.76	19.20	13.04	8.31
		Protective	20.47	17.54	9.63	8.80	19.04	18.66	12.73	5.50
7.	*Lawsonia inermis*	Curative	21.82	14.43	8.74	5.82	20.63	17.40	11.83	6.64
		Protective	21.43	10.81	7.72	4.51	19.71	14.21	9.73	4.91
8.	*Punica granatum*	Curative	21.63	14.72	7.64	4.82	21.33	17.41	10.81	4.71
		Protective	21.00	12.84	6.74	3.45	20.60	16.11	10.21	3.53
9.	*Rhizophora apiculata*	Curative	19.43	11.51	6.84	3.91	20.82	11.01	9.71	4.00
		Protective	18.00	10.71	6.24	3.11	18.64	10.01	8.64	3.10
10.	*Tamarinda indicus*	Curative	21.30	13.63	7.43	5.43	21.61	18.14	11.52	4.54
		Protective	20.43	11.32	7.30	3.54	20.63	15.70	9.62	3.60
	Streptomycin (100)			0.0				0.0		
	Control			22				22		
	S.E.		0.01	0.01	0.001	0.01	0.02	0.01	0.02	0.01
	C.D. (P=0.05)		0.03	0.02	0.006	0.02	0.04	0.02	0.05	0.03

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