

In-vitro* Screening of Plant Extract to Control Bacterial Leaf Blight of Rice Disease Caused by *Xanthomonas oryzae* pv *oryzae

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Abstract The antibacterial potential of various plant extracts as alternative to chemical pesticides for controlling bacterial leaf blight of rice was investigated. Ten different species of plants were collected from surrounding of RAU, Pusa (Bihar). Three concentrations of plant extracts 5, 10 and 15% were used to test the antibacterial activity of plant extract by using poison food technique and were used as seed treatment to know the enhancement of seed germination and seedling vigor. All the ten plant extracts were found to inhibit the growth of *Xanthomonas oryzae* pv *oryzae*, *in-vitro*, at all three concentrations as compared to control. When compared with standard check, streptomycin sulfate (100 ppm), *Ocimum tenuiflorum* and *Calotropis procera* were at par at 5% concentrations of plant extracts, whereas at 10% concentration *Ocimum tenuiflorum*, *Calendula officinalis*, *Zingiber officinale* and *Allium sativum* were highly effective and at par among themselves and at 15% concentrations, *Calendula officinalis*, *Zingiber officinale*, *Allium sativum*, *Ocimum tenuiflorum* showed promising effect or at par with streptomycin sulfate (100 ppm). All the plant extracts increased seed germination and seedling vigor of rice

varieties as compared to control except *Allium cepa*.

Keywords *Xanthomonas oryzae* pv *oryzae*, Bacterial leaf blight, Plant extract, Rice.

Introduction

Rice (*Oryza sativa* L.) is one of the most widely cultivated food crops in the world. Losses due to pests and diseases are important constraints in rice production. Seed borne bacterial leaf blight (BLB) of rice, caused by *Xanthomonas oryzae* pv *oryzae* (Ishiyama) [1], is the major limiting factor in rice seed production. In India, the yield loss due to this disease is upto 81.3% [2, 3]. In addition to direct yield loss in the seed production programs, this disease also adversely affects the seed quality through seed discoloration. This pathogen is known to be seed-borne and seed transmitted [3]. However, the primary sources of the inoculum are external and internal contaminated seeds [4]. The results of the contaminated seed are poor germination and severe infection affects the plants at tillering and flowering stages, thereby resulting in the formation of chaffy seeds.

Several broad spectrum bactericides have been recommended for the bacterial leaf blight. However, the chemicals are expensive, it affect the environment, consumers health and beneficial micro-organism. Plant origin biocides are non phyto-toxic, systemic and easily biodegradable [5—7]. The antibacterial activities of different plant extracts against plant disease have been previously investigated [8].

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Table 1. Plant species used in the study.

Sl. No.	Plant extracts	Family	Part of plant used
1	Marigold (<i>Calendula officinalis</i>)	Calenduleae	Leaves
2	Tulsi (<i>Ocimum tenuiflorum</i>)	Lamiaceae	Leaves
3	Akwani (<i>Calotropis procera</i>)	Asclepiadaceae	Leaves
4	Dathura (<i>Datura stramonium</i>)	Solanaceae	Leaves
5	Chirchiri (<i>Achyranthes aspera</i>)	Amaranthaceae	Leaves
6	Onion (<i>Allium cepa</i>)	Amaryllidaceae	Bulb
7	Ginger (<i>Zingiber officinale</i>)	Zingiberaceae	Rhizome
8	Garlic (<i>Allium sativum</i>)	Amaryllidaceae	Cloves
9	Neem (<i>Azadirachta indica</i>)	Meliaceae	Leaves
10	Bhang (<i>Cannabis sativa</i>)	Cannabaceae	Leaves

Therefore, in the present study, an attempt has been made to evaluate plant extracts in place of synthetic chemicals not only to reduce the disease incidence due to *X. oryzae* pv *oryzae* but also to improve the seed quality. Ten different plant species were screened for their antibacterial activity against *X. oryzae* pv *oryzae* under *in vitro* condition and their effect on seed germination and seedling vigor were also investigated.

Materials and Methods

Plant material

All the plant materials of ten plant species tested for their antibacterial activity were collected from surrounding area/region, RAU, Pusa, Samastipur (Bihar), India (Table 1).

Plant extracts preparation

Fresh leaves, bulb, cloves and rhizomes of ten different plants were collected and washed in tap water 3 to 4 times, then distilled water. 100 grams of leaves, bulb, cloves and rhizomes of each were ground in the mixture juicer-cum-grinder by adding 100 ml sterilized water @ 1:1 (W/V) and it was filtered through muslin cloth. The plant extract so prepared were filtered through sterilized filter paper (44 micron meter).

The extract was considered as 100% pure concentration, further dilution (5, 10 and 15%) were made for testing its efficacy (antibacterial property) by poison food technique.

In vitro screening of plant extract against *X. oryzae* pv *oryzae*

For screening of antibacterial activity of the plant extracts, different concentration (5, 10 and 15%) were obtained by adding appropriate amount of standard solution of plant extracts to the nutrient sucrose agar (NSA) medium in conical flask, then pored in petriplates (replicated thrice for each treatment). The solidified petriplates were spot inoculated with a loop of 48 hour old *X. oryzae* pv *oryzae* culture and kept for incubation at 28±1°C for 48 hour. After incubation, the observations were recorded for the growth of *X. oryzae* pv *oryzae* at different concentration of plant extracts. NSA without leaf extracts served as control and NSA with streptomycin sulfate (100 ppm) served as the standard check.

Effect of plant extracts on seed germination and seedling vigor

The paddy seeds varieties i.e. Pusa Basmati and Rajendra Bhagwati were soaked with crude aqueous extracts overnight at room temperature and used in paper towel method to check the seed germination and seedling vigor. Four replicates of 100 seeds were maintained for each treatment, while distilled water treated served as control.

Results and Discussion

The effects of natural products (plant extracts) in comparison with bactericide (Streptomycin sulfate) were evaluated against bacterial leaf blight pathogen, *X. oryzae* pv *oryzae* and presented in Table 2.

Table 2. Effect of different plant species aqueous extracts on the growth of *X. oryzae* pv *oryzae* by poison food technique at 5, 10 and 15% concentration.

Sl. No.	Plant extract	5% Concentration		10% Concentration		15% Concentration	
		Bact growth measured (cm)	% inhibition over control	Bacterial growth measured (cm)	% inhibition over control	Bacterial growth measured (cm)	% inhibition over control
1	<i>Calendula officinalis</i>	0.300	63.98	0.200	75.99	0.183	78.04
2	<i>Ocimum tenuiflorum</i>	0.283	66.02	0.200	75.99	0.200	75.99
3	<i>Calotropis procera</i>	0.283	66.02	0.250	69.99	0.233	72.03
4	<i>Tulsi stramonium</i>	0.333	60.02	0.316	62.07	0.266	68.07
5	<i>Achyranthes aspera</i>	0.366	56.07	0.233	72.03	0.216	74.07
6	<i>Allium cepa</i>	0.416	50.06	0.383	54.03	0.366	56.07
7	<i>Zingiber officinale</i>	0.316	62.07	0.216	74.07	0.183	78.04
8	<i>Allium sativum</i>	0.500	39.97	0.216	74.07	0.183	78.04
9	<i>Azadirachta indica</i>	0.350	57.98	0.283	66.03	0.266	68.07
10	<i>Cannabis sativa</i>	0.383	54.02	0.250	69.99	0.233	72.03
11	Control	0.833		0.833		0.833	
12	Streptomycin sulfate (100 ppm)				0.205		
	SE±	0.025		0.022		0.019	
	CD (5%)	0.073		0.067		0.057	

All the ten plant extracts were found to inhibit the growth of *X. oryzae* pv *oryzae* as compared to control at all the three concentrations i.e. 5, 10 and 15%. The effect of plant extracts increased with increase in concentration of plant extracts and percent inhibition over control were presented in Table 2.

At 5% concentration highest % of inhibition over control were recorded in the *Ocimum tenuiflorum*, *Calotropis procera* followed by *Calendula officinalis* and *Zingiber officinale* over control. However, at 10% concentration *Ocimum tenuiflorum*, *Calendula officinalis* followed by *Zingiber officinale*, *Allium sativum* and *Achyranthes aspera*. In case of 15% concentration *Zingiber officinale*, *Calendula officinalis*, *Allium sativum* performed best followed by *Ocimum tenuiflorum* and *Achyranthes aspera*.

When compared with standard check, streptomycin sulfate (100 ppm) *Ocimum tenuiflorum* and *Calotropis procera* were at par at 5% concentrations of plant extracts, where as at 10% concentration *Ocimum tenuiflorum*, *Calendula*, *Zingiber officinale* and *Allium sativum* showed best or at par and at 15% concentrations *Calendula officinalis*,

Zingiber officinale, *Allium sativum*, *Ocimum tenuiflorum* showed best or at par with streptomycin sulfate (100 ppm) (Table 2).

Effects of plant extracts on seed germination and seedling vigor

All the plant extracts, found effective against the BLB pathogen, were tested for their effect on seed germination and seedling vigor of rice by seed treatment of different varieties i.e. Pusa Basmati and Rajendra Bhagwati.

The results presented in Table 3 showed that seed treatment with plant extracts enhanced the seed germination and seedling vigor as compared to control, except onion. It also enhanced the root and shoot length in all the tested varieties.

The seed treatment with *Zingiber officinale* was found most effective among all the tested plant extracts. It showed highest germination i.e. 90 and 86% and seedling vigor i.e. 1542.60 and 1193.68, as compared to control i.e. 82 and 78% germination and 619.92 and 758.16 vigor index in both the rice varieties P. Basmati and R. Bhagwati respectively.

Table 3. Effect of plant extract as seed treatment on seed germination and seedling vigor of rice. Germn % : Germination percentage, MSL : Mean shoot length, MRL : Mean root length, VI : Vigor index.

	Plant extract		Rice varieties	
			P. Basmati	R. Bhagwati
1.	Bhang (<i>Cannabis sativa</i>)	Germn %	83	79
		MSL	4.10	5.64
		MRL	4.40	4.62
		VI	705.5	810.54
2.	Chirchiri (<i>Achyranthes aspera</i>)	Germn %	88	79
		MSL	4.10	5.12
		MRL	4.10	4.74
		VI	721.60	778.94
3.	Dathura (<i>Datura stramonium</i>)	Germn %	89	79
		MSL	4.84	5.10
		MRL	5.20	5.46
		VI	893.56	834.24
4.	Tulsi (<i>Ocimum tenuiflorum</i>)	Germn %	90	81
		MSL	4.28	6.34
		MRL	4.70	5.34
		VI	808.20	946.08
5.	Onion (<i>Allium cepa</i>)	Germn %	81	76
		MSL	3.60	5.54
		MRL	3.20	3.54
		VI	550.8	690.08
6.	Marigold (<i>Calendula officinalis</i>)	Germn %	84	81
		MSL	4.20	5.90
		MRL	5.30	4.64
		VI	798	853.74
7.	Neem (<i>Azadirachta indica</i>)	Germn %	83	79
		MSL	4.38	6.90
		MRL	5.50	4.70
		VI	820.04	916.40
8.	Akwan (<i>Calotropis procera</i>)	Germn %	84	79
		MSL	4.20	6.10
		MRL	4.34	4.60
		VI	717.36	845.30
9.	Garlic (<i>Allium sativum</i>)	Germn %	84	79
		MSL	2.92	6.10
		MRL	4.76	3.90
		VI	645.12	790
10.	Ginger (<i>Zingiber officinale</i>)	Germn %	90	86
		MSL	9.90	6.30
		MRL	7.24	7.58
		VI	1542.60	1193.68
11.	Control	Germn %	82	78
		MSL	4.00	5.20
		MRL	3.56	4.52
		VI	619.92	758.16

The results revealed that in case of untreated seed, the seed germination and seedling vigor was poor. When treated with plant extracts enhancement was observed. Application of plant extracts prior to seed germination was found to be better in improving the plant vigor, shoot and root length.

In the present study, ten different plant species which belong to different families were tested for antibacterial activity against bacterial leaf blight pathogen, *X. oryzae* pv *oryzae*. The leaf extract at 15% concentration was more effective in suppression of *X. oryzae* pv *oryzae*, growth in comparison to the con-

trol. When compared to standard check streptomycin sulfate (100 ppm) the *Zingiber officinale*, *Ocimum tenuiflorum*, *Calendula officinalis*, *Allium sativum* showed more antibacterial activity against *X. oryzae* pv *oryzae*, at 15% concentration of plant extracts. However, the leaf extract of remaining plant species showed lower antibacterial activity compared to control and standard check i.e. streptomycin sulfate (100 ppm).

Various plant extracts were reported to possess differing level of antimicrobial activities *in vitro* against phytopathogenic fungi, yeast, and bacteria. However, only few limited data available on antibacterial activity due to plant extracts against plant pathogenic bacteria.

Earlier, Kagle et al. [7] and Narasimhan et al. [9] reported antibacterial activity and management of bacterial diseases with use of different plant extracts in other crops.

Kumar et al. [10] also reported that at 25% concentration minimum number of colonies developed in case of *Eucalyptus grandifolia* (10.00) followed by *Curcuma longa* (13.00) and *Ocimum sanctum* (18.67) which showed maximum inhibition i.e. 68.41, 58.94 and 41.06% respectively.

Rukhsana Jabeen [11] screened twenty five different species of medicinal plants against *X. oryzae* pv *oryzae*. Out of twenty five medicinal plants, *Thuja orientalis* (cone and leaves), *Azadirachta indica* (seeds and fruits), *Amomum subulatum* (fruits), *Terminalia chebula* (fruits), *Anethum graveolens* (fruits) and *Ferula assa-foetida* (fruits) decoctions showed significant activity.

Govindappa et al. [12] reported that *Adathoda vasica* leaf extract showed antibacterial activity against *X. oryzae* pv *oryzae* and ability to enhance seed germination and seedling vigor and further demonstrated that the *A. vasica* leaf extract had the ability to induce the activation of defence enzymes accumulation which can be associated with induction of resistance against rice bacterial leaf blight.

Present finding is also similar with the findings

of Kagale et al. [7], Meena and Gopalakrishnan [13] and Sunder et al. [14]. They also reported the inhibitory effect of *Azadirachta indica*, *Curcuma longa*, *Datura metel* and *Zingiber officinale* against *X. oryzae* pv *oryzae*.

The plant extracts i.e. *Zingiber officinale*, *Ocimum tenuiflorum*, *Calendula officinalis* and *Allium sativum* showed more antibacterial activity against *X. oryzae* pv *oryzae* compared to control and standard check i.e. streptomycin sulfate (100 ppm), also enhanced the seed germination and seedling vigor of rice varieties except onion.

Therefore, the use of *Zingiber officinale*, *Ocimum tenuiflorum* and *Calendula officinalis* in order to control seed borne bacteria, *X. oryzae* pv *oryzae* and development of disease. It is effective and environmentally safe in the management of bacterial leaf blight disease in rice.

References

- Swings J, Van Den Mooter M, Vauterin L, Hoste B, Gills M, Mew TW, Kersters K (1990) Reclassification of causal agents of bacterial blight (*Xanthomonas campestris* pv *oryzae*) and bacterial leaf streak (*Xanthomonas campestris* pv *oryzae*) of rice as pathovars of *Xanthomonas oryzae* (ex Ishiyama 1922) sp. Nov, nom Rev. Int J Sys Bacteriol 40 : 309—311.
- Gnanamanickam SS, Priyadarshani B, Narayanan NN, Vasudevan P, Kavitha S (1999) An over view of bacterial leaf blight disease of rice and strategies for its management. Curr Sci 77 : 1435—1444.
- Veena MS, Shetty HS, Mortensen CN, Mathur SB (2000) Bacterial leaf blight of rice. Tech Bull, Dep Studies in Appl Bot, Univ Mysore, India and Danish Govt Inst Seed Pathol for Develop Countries, Denmark, pp 16.
- Srivastava DN, Rao YP (1964) A single technique for detecting *Xanthomonas oryzae* in rice seeds. Seed Sci Technol 5 : 123—127.
- Mason JR, Mathew DN (1996) Evaluation of neem as a bird repellent chemical. Int J Pest Manag 42 : 47—49.
- Singh DC (1994) Scope of medicinal and aromatic plants in pest management. Int Symp, Allelopathy in Sustainable Agriculture, Forestry and Environment, New Delhi 68 : 6—8.
- Kagle S, Marimuthu T, Thayumanavan B, Nandakumar R, Samiyappan R (2004) Antibacterial activity and induction of systemic resistance in rice by leaf extract of *Datura metel* against *Rhizoctonia solani*

- and *Xanthomona oryzae* pv *oryzae*. Phys Mol Pl Pathol 65 : 91—100.
8. Okigbo RN, Nmeka IA (2005) Control of yam tuber rot with leaf extracts of *Xylopi aethiopica* and *Zingiber officinale*. Afr J Biotechnol 4 : 804—807.
 9. Narasimhan V, Selvam R, Marriappan V (1995) In: Mariappan 5 editor. Neem for the management of crop diseases. New Delhi, Assoc Publ Co, pp 15—121.
 10. Kumar M, Parate RL, Ninawe BN (2009) Effect of botanicals, bioagents and some chemicals against *Xanthomonas oryzae* pv *oryzae*. J Pl Dis Sci 4 : 60—63.
 11. Rukhsana Jabeen (2011) Medicinal plants—a potent antibacterial source against bacterial leaf blight (BLB) of rice. Pak J Bot 43 : 111—118.
 12. Govindappa M, Umesha S, Lokesh S (2011) *Adathoda vasica* leaf extract induces resistance in rice against bacterial leaf blight disease (*Xanthomonas oryzae* pv *oryzae*). Int J Pl Physiol and Biotechnol 3 : 6—14.
 13. Meena C, Gopalakrishnan J (2004) Efficacy of plant extracts against bacterial blight of rice. Ann Pl Prot Sci 12 : 334—346.
 14. Sunder S, Singh R, Dodan DS (2005) Management of bacterial blight of rice with botanical extracts and non-conventional chemical. Pl Dis Res 20 : 12—17.