

Original Research Article

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***In vitro* Evaluation of Various Fungicides and Plant Extracts against *Xanthomonas oryzae* pv. *oryzae* Isolated from Rice (*Oryza sativa* L.)**

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ABSTRACT

The bacterial leaf blight of rice caused by *Xanthomonas oryzae* pv. *oryzae* is the disease of great economics importance in India especially in Bihar. Management of BLB disease can play a vital role in management of rice disease. In the present investigation evolution of different plant extract (botanical) and new chemical on growth of bacteria by poison food technique and sowing by best botanical and new chemical were conducted and antagonistic activity were tested. Three botanical and three new chemical were screened in-vitro for antagonistic activity agent BLB. Among six tested botanical and new chemical isolated again *Xanthomonas oryzae* pv. *oryzae*, The aqueous leaf extract of botanicals were effective i.e. *Allium sativum*, *Azadirachta indica* and *Zingiber officinale* were found to be antagonistic towards *Xanthomonas oryzae* pv. *oryzae* @ 15% conc. after 48 hours. Three chemicals found effective in reducing severity of BLB were Blitox-50+Streptocycline, Ergon 44.3 SC and Nativo 75WG @50 ppm conc. showed inhibition colony width growth of pathogen by poisons food technique respectively. The seed treatment with six effective i.e. three botanical and three new chemical provide good protection of seed against seed borne pathogen resulting increase seed germination and seedling vigor of rice variety as compare to control.

Keywords

Rice, *Xanthomonas oryzae*, BLB, Botanical and Fungicide.

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Introduction

Bacterial leaf blight of rice caused by *Xanthomonas oryzae* pv. *oryzae* (Ishiyama) Swing *et al.*, (1990) is the disease of great economic in India (Bihar). BLB disease prevost in almost all the paddy growing region in the state, it is considered to be a major constraint for low rice productivity in India (Bihar). It was first reported from India by Srinivasm *et al.*, (1959) from Maharashtra. 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 long lasting and destructive phase. Some of the chemical are effective against this disease but they have not proved economical and harmful residues in soil and plant. Earlier investigation was planned to manage the disease with the help of botanical and new chemical which have antagonistic bacterial activity. Antagonistic activity of botanical and new chemical was judged against premising isolates of BLB by Nutrient Agar well plated method (Manav and Thind, 2002). Botanical and new chemical control of BLB can play a vital role in

management rice disease (Singh *et al.*, 2010). In the present investigation, different botanical and new chemical were control by poison food technique and sowing of most efficient botanical and new chemicals were conducted and tested the antagonistic activity against the BLB pathogen *Xanthomonas oryzae* pv. *oryzae*.

There are also some reports that seed treatment with botanical and new chemicals influences the germination of seed and growth of seedling in several crops (Sen, 1999, Biswas *et al.*, 2008). Therefore, the present study was undertaken to find out the activity of botanicals and new chemicals isolates as seed treatment on seed germination four rice variety i.e. Pusa Basmati.

Materials and Methods

Isolation and identification of causal organism

The leaves from the diseased plants were collected from the field and cut into small pieces along with healthy portion. Cut pieces were sterilized by the surface disinfectants e.g. 0.1% mercuric chloride for 30 seconds. After sterilization the cut pieces were washed three times with sterile water.

The cut pieces were then placed on sterile blotter paper to remove excess water. The cut pieces were then placed on the Nutrient Agar plate. The plate were labelled and placed in the incubation chamber for 7 days at 25 + 2° C. After 7 days of incubation, the fungi grown on culture media. In bacterial leaf blight causal organism *Xanthomonas oryzae* isolated on nutrient Agar media. A portion of culture was taken by inoculating needle on nutrient Agar media plates. The pathogen, thus purified, was kept in refrigerator for future use. All these operations were done aseptically in the laminar air flow chamber.

Evaluations of various plant extracts and new chemicals on growth of bacteria by poisoned food technique

All the plant materials of five plant species tested for their antibacterial activity were collected from surrounding area, RAU, Pusa, Samastipur (Bihar), India. Fresh leaves i.e. *Calendula officinalis*, *Ocimum tenuiflorum* and *Azadirachta indica*, cloves *Allium sativum* and rhizome *Zingiber officinale* of five different plants were evaluated against BLB to examine their inhibitory efficacy on growth of the bacteria. All botanical leaf plant were collected and washed 3-5 times by tap water than after washed with distilled water, processed with sterile distilled water at 1:2 ratio viz: 1g tissue in 2ml water then grinding in mixing grinder, each of separately then filtered with muslin cloth. Filtered was centrifuged at 1000spm for 15 min. and clear supernatants was collected. The supernatant was filtered, sterilized to avoid contamination. The obtained extract formed the standard extract solution.

New chemical

Nativo (Trifloxystobin + Tebuconazole), Ergon (Kerosim methyl), Mirador (Azoxytobin) and Blitox-50 (Copper-oxychloride) + Streptocycline. Four concentration of each chemical i.e. 10 ppm, 25 ppm and 50 ppm were used against the pathogen.

To obtain desired concentration of botanical extract and new chemical in the medium on amount of stock solution to be added in NA (Nutrient agar) medium. The all material were incorporated into NA medium at desire concentration. The amended NA was poured into 20 mm sterilized petriplated under aseptic condition in inoculums chamber @ 20ml medium/plate and NA without leaf extract and new fungicide served as control

and allowed to solidify. All petriplates were inoculated a loop full of 48 hrs. old *Xanthomonas oryzae* pv. *oryzae* culture were streaked on these prepared plates and kept for incubation at 28.c for 72 hrs. After incubation, the observations were recorded on the growth of *Xanthomonas oryzae* pv. *oryzae*. on different concentration. Based on observation, % inhibition was calculated by following formula.

$$\text{Percentage Inhibition (PI)} = \frac{C-T}{C} \times 100$$

C = Radial growth of pathogen in control plate.
T = Radial growth of pathogen in dual culture

Effect of seed treatment with most efficient botanical and new chemical *in vitro* was done

The seed were soaked with botanical extracts and new chemical (i.e. effective against the BLB pathogen). Seed of variety i.e. Pusa Basmati were soaked with botanical and new chemical and grown in sterillized petriplates. Incubate the plate initially for 24 hrs. under controlled condition in growth chamber. Five replicates of 25 seeds were maintained for each treatment, while distilled water treated served as control.

Results and Discussion

We tested five different plant species for antibacterial activity of bacterial leaf blight pathogen. All the five aqueous botanicals extracts i.e. *Calendula officinalis*, *Ocimum tenuiflorum*, *Zingiber officinale*, *Azadirachta indica* and *Allium sativum* inhibit the growth of *Xanthomonas oryzae* pv.*oryzae* as compare to control in all the three concentrations i.e. 5, 10 and 15%. The effect of plant extracts increases with increase in concentration of plant extracts.

When compared with out of five botanicals after 48 hours of incubation *Allium sativum* (87.33%), *Azadirachta indica* ((71.23%) and *Zingiber officinale* (60.83%) were found to be antagonistic towards *Xanthomonas oryzae* pv.*oryzae* @ 15% conc. after 48 hours. Narasimhan *et al.*, (1995) and Kagle *et al.*, (2004) have reported antibacterial activity and management of bacterial diseases with use of different plant extracts in other crops. Kumar *et al.*, (2009) also reported that at 25 % concentration minimum number of colonies development were observed in case of *Allium sativum* (18.67) which showed maximum inhibition i.e. 41.06%, Present finding also similar with the findings of Kagale *et al.*, (2004), Meena and Gopalakrishnan (2004) and Sunder *et al.*, (2005). They also reported the inhibitory effect of *Azadirachta indica* and *Zingiber officinale* against *Xanthomonas oryzae* pv. *oryzae*.

Evaluation of different new chemicals against *Xanthomonas oryzae* pv. *oryzae*

An experiment was conducted the effect of four new chemicals namely Nativo 75WG, Blitox-50+Streptocycline, Mirador and Ergon 44.3 SC @10ppm, 25ppm and 50ppm by poisoned food technique. The colony width growths of the pathogen were recorded after 48 hrs. at 27°C.

When compared with all new chemicals after 48 hours of incubation Blitox-50 + Streptocycline (70.25%), Ergon 44.3 SC (66.4%), Nativo 75WG (49.91%) and Mirador (35.33%) which against *Xanthomonas oryzae* pv. *oryzae*. (%). Patel *et al.*, (2009) concerned that Blitox-50 is superior treatment for reducing Bacterial leaf blight intensity and incur grain and show yield and it is highly effective. Where also same reported by previous worker (Munna *et al.*, 2009 and Thimmegowda.

The most efficient botanical (Plant leaf extract) and new chemical will be evaluated for seed treatment (germination) of rice

Seed treatment with these three plant extracts *Allium sativum*, *Azadirachta indica* and *Zingiber officinale* Neem, effective against the *Xanthomonas oryzae* pv. *oryzae* were tested for seed germination of rice through seed treatment on variety i.e. Pusa Basmati @ different conc. i.e. 5%, 10% and 15% after 48 hours. Aqueous leaf/bulb/cloves extracts @ of different plants were increased the shoot

length of rice variety. Seed treatment with *Zingiber officinale*, *Azadirachta indica* and *Allium sativum* @ 15% conc. resulted in 88%, 85% and 72% germination respectively.

Allium sativum, *Azadirachta indica* and *Zingiber officinale* enhanced the root and shoots length and seed germination in cv. Pusa Basmati. Kagle *et al.*, (2004), Kumar *et al.*, (2009) and Narasimhan *et al.*, (1995) recommended the use of garlic, ginger as well as neem in order to get high germination and control seed borne bacteria.

Table.1 *In vitro* effect of different aqueous extracts of botanicals on the growth of *Xanthomonas oryzae* pv. *Oryzae*

Treatment	5% Concentration		10 % Concentration		15% Concentration	
	Bacterial Growth colony width (mm) *	Per cent inhibition over control	Bacterial Growth colony width (mm) *	Per cent inhibition over control	Bacterial Growth colony width (mm) *	Per cent inhibition over control
<i>Calendula officinalis</i> (Marigold)	16.375	45.42	12.025	59.92	11.325	62.25
<i>Ocimum tenuiflorum</i> (Tulsi)	15.025a	49.92	11.663	61.12	11.250	62.50
<i>Zingiber officinale</i> (Ginger)	12.900	57.00	12.125	59.58	11.750	60.83
<i>Azadirachta indica</i> (Neem)	12.375	58.75	9.200	69.33	8.625	71.25
<i>Allium sativum</i> (Garlic)	8.300	72.33	5.050	83.17	3.800	87.33
Control	30.000		30.000		30.000	
CD @5%	1.070		1.536		1.502	
CV %	4.517		7.690		7.843	

Table.2 *In vitro* effect of different new chemicals on the growth of *Xanthomonas oryzae* pv. *Oryzae*

Treatment	10 ppm		25 ppm		50 ppm	
	Bacterial Growth colony width (mm)*	Per cent inhibition over control	Bacterial Growth colony width (mm)*	Per cent inhibition over control	Bacterial Growth colony width (mm)*	Per cent inhibition over control
Nativo 75WG (Trifloxystrobin 25%+Tebuconazole 50%)	18.375	38.75	16.675	44.41	15.025	49.91
Blitox-50 (Copper oxychloride+Streptocycline)	11.000	63.33	10.375	65.41	8.925	70.25
Mirador (Azoxystobin)	22.425	25.25	21.475	28.42	19.400	35.33
Ergon 44.3 SC (Kreosim methyle)	14.175	52.75	13.700	54.33	10.075	66.41
CONTROL	30.000		30.000		30.000	
CD @5%	2.038		1.203		1.640	
CV %	6.980		4.288		6.463	

Table.3 Effect of Plant extract as seed treatment on seed germination of rice

Plant Extract	5 % Concentration			10 % Concentration			15 % Concentration		
	Germination* %	Shoot length* (cm)	Root length* (cm)	Germination* %	Shoot length* (cm)	Root length* (cm)	Germination* %	Shoot length* (cm)	Root length* (cm)
<i>Azadirachta indica</i> (Neem)	71	3.95	4.22	79	4.08	4.49	85	4.18	4.62
<i>Allium sativum</i> (Garlic)	56	2.26	4.36	63	2.48	4.53	72	2.76	4.64
<i>Zingiber officinale</i> (Ginger)	75	9.26	6.99	81	9.41	7.09	88	9.78	7.19
Control	64	3.78	3.30	64	3.78	3.30	64	3.78	3.30
CD (5%)	4.81	0.31	0.20	4.54	0.30	0.15	4.40	0.19	0.11
CV%	10.99	4.80	3.23	10.68	0.33	2.36	9.81	2.85	1.71

Fig.1 Effect of plant extracts as seed treatment on seed germination of Rice



Fig.2 Effect of chemicals as seed treatment on seed germination of rice



Effect of seed treatment with new chemicals on seed germination

In respect to new chemicals, for better germination was recorded seed treatment with Blitox-50 +Streptocycline, Nativo 75WG and Ergon 44.3 SC effective against the BLB pathogen were tested for seed germination of rice through seed treatment on variety i.e. Pusa Basmati @ 10ppm, 25ppm and 50ppm. New chemicals were increased the shoot length of rice varieties. It also enhanced the root and shoot length in rice variety i.e. Pusa Basmati seed treatment with new chemicals i.e. Blitox-50 +Streptocycline (88%), Nativo 75WG (80%) and Ergon 44.3 SC (72%) @ 50 ppm conc.that effective against the BLB pathogen enhanced the seed germination as compare to control.

Present finding also similar with the findings of Patel *et al.*, (2009), Munna *et al.*, (2004) and Thimmegowda *et al.*, (2005). They also reported the Blitox-50+ streptocycline, increasing grain and inhibitory effect against *Xathomonas oryzae* pv. *oryzae*.

So, Seed treatment with botanical and new chemicals viz: *Zingiber officinale*, *Azadirachta indica* Blitox-50 +Streptocycline (88%), Nativo 75WG respectively isolates was effective in increasing seed germination and seed germination of Pusa Basmati rice variety as compare to control. So, those two botanicals as well as two new chemicals were used for further studies in field conditions.

It is concluded in present investigation, all the tested botanical and new chemical isolated

again *Xanthomonas oryzae* pv. *oryzae*, The aqueous leaf extract of botanicals were effective i.e., *Allium sativum*, *Azadirachta indica* and *Zingiber officinale* were found to be antagonistic towards *Xanthomonas oryzae* pv. *oryzae* @ 15% concentration after 48 hours. Three chemicals found effective in reducing severity of BLB were Blitox-50+Streptocycline; Ergon 44.3 SC and Nativo 75WG @50 ppm concentration showed inhibition colony width growth of pathogen by poisons food technique respectively. The seed treatment with six effective i.e. three botanical and three new chemical provide good protection of seed against seed borne pathogen resulting increase seed germination and seedling vigor of rice variety as compare to control.

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