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Short Communication

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EVALUATION OF CHEMICALS AGAINST BACTERIAL BLIGHT OF RICE CAUSED BY *XANTHOMONAS ORYZAE* PV. *ORYZAE*

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Abstract

The experiemant was conducted at Bangladesh Rice Research Institute to manage the bacterial blight disease of rice caused by *Xanthomonas oryzae* pv. *oryzae*. Efficacy of eight chemicals (Cuproxat 345 SC, BB Stopper 20% SC, Tetrax R 500, Acizol 20, Thiasol 20, Krosin AG 10 SP, Dibacteria 10 SP and Sunbenchlor 37 WP) with two doses (recommended and double doses) and two application time (preventive and curative) were tested in pot experiment under nethouse and field conditions. Streptomyc in based bactericides Krosin AG 10 SP and Dibacteria 10 SP showed strong antibacterial activity *in vitro* following disc diffusion method but they showed dissimilar result in nethouse and field condition. The lowest lesion length (9.7 cm), infected leaf area (9.3%) and disease index (30.56) were found when applied either single or double doses of Thiadiazole based chemical Thiasol 20, where as in control plot these parameters showed the maximum values of 32.46 cm, 85.17% and 100, respectively. But the effectiveness of this chemical was not satisfactory as much as for registration. Though *in vitro* condition some chemicals showed very good suppression effect. Finally none of the chemicals were found effective against bacterial blight under net house or field conditions. Effect of the tested chemicals on yield and yield contributing characters in field trial were not consistent.

Introduction

Bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) considered as a most destructive disease occurs in all Agro Ecological Zones (AEZ) of Bangladesh and mostly in two rice growing seasons namely, Aman (June-July to November-December) and Boro (November-December to April-May) (Miah, 1973; Miah *et. al.*, 1985; Khan *et. al.*, 2009; Latif *et. al.*, 2011) and causes considerable yield loss. Bacterial blight (BB) can occurs at all the growing stages of rice and is known by either leaf blight or kresek symptoms. It is also considered as an important disease in most of the South and South-East Asian countries (Sharma 1991). Bacterial blight of rice is one of the oldest disease of rice, which was first

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noticed by the farmers of Japan in 1884 (Mizukami, 1956; Tagami and Mizukami, 1962) and considered as a limiting factor for rice production because of its high epidemic potential (Khan *et. al.*, 2010; Verdier *et. al.*, 2012; Xia *et. al.*, 2012). Yield loss due to mild infection has been recorded about 10-12% (Kuiper *et. al.*, 1989) and in Japan (Soga 1918), India (Srivastava *et. al.*, 1966), Bangladesh (Shahjahan, 1993) and Pakistan (Khan *et. al.*, 2015) around 50, 60, 30 and 57% rice yield reductions has been reported, respectively. The yield loss depends on the stages of the crop attack, degree of cultivar susceptibility and to a great extent, on conduciveness of the environment in which it occurs (Reddy *et. al.*, 1978). Heavy rainfall, high humidity and temperature, flood and stormy weather provide favorable conditions for high incidence and severity of BB (Soga 1918, Fujikawa *et. al.*, 1957; Mizukami and Wakimoto, 1969; OCTA, 1970).

In Bangladesh, around 80% of 13.9 million hectares (Mha) total cropped area are occupied by the rice. Currently, rice alone constitutes around 92% of the total annual food grains production of the country and it provides around 75% of the total calories along with 55% of the protein in average daily diet of Bangladeshi people (Bhuiyan *et. al.,* 2002). It has been estimated that population of Bangladesh will reach 215.4 million in 2050, when 44.6 MT of clean rice will be required (Kabir *et. al.*, 2015). To increase the rice yield, high yielding variety (HYV) are introducing day by day and higher dose of nitrogen fertilizer requirement in HYV encourages the occurrence and severity of BB in the field (Kim and Cho, 1970; Kauffman, 1972; Chattopadhyay and Mukharjee, 1973; Mohanty *et. al.,* 1983; Devadath *et. al.,* 1987). In Bangladesh, a wide yield gap is resulted due to various factors where pest especially diseases are one of the most important threat which leading to disastrous consequences (Hossain, 2001). To date, out of 31 diseases of rice; ten diseases are considered as major in Bangladesh (Miah *et. al.*, 1981; Latif *et. al.*, 2007) and among them bacterial blight has been considered as important one because of its wide spread occurrence and significant damage potential (*Khan et. al.*, 2010). The severity and significance of damages caused by infection have necessitated the development of strategies to control and manage the disease. Although the use of Bordeaux mixture, antibiotics and other copper as well as mercurial compounds were reported in early fifties but effective chemical control that can be used in different rice eco-systems is still not available against this intractable disease (Devadath, 1989). Thus, the management of bacterial plant diseases remains a formidable 'grand challenge' for plant pathologists. In Bangladesh, there is no recommended bactericides that can be used to control BB effectively under field condition (Khan *et. al.*, 2010). In the present study an attempt was made to evaluate the effectiveness of some unregistered chemicals as bactericides for the control of bacterial blight of rice.

Materials And Methods

Treatments

The experiment was carried out both in the net house and experimental field (West Byde) of Plant Pathology Division, Bangladesh Rice Research Institute (BRRI), Gazipur, during T. Aus (April-September), 2011 season. A bacterial blight susceptible rice variety 'Purbachi', was used as test plant. A bacterial blight isolate BXO9 was obtained from the Plant Pathology Division, BRRI was used as inoculum for artificial inoculation. The isolate is most virulent and reference isolate of major race pathogen in Bangladesh (Khan *et. al.*, 2009).

Table 1. Bactericides tested in nethouse and field conditions

Commercial name	Active ingredient	Dose for 1 ha area
Cuproxat 345 SC	Tribasic Copper Sulphate	1000 ml
Cuproxat 345 SC		2000 ml
BLB Stopper 20% SC	Thiodiazole Copper	1200 ml
BLB Stopper 20% SC		2400 ml
Tetrax R 500	Tetracycline HCl BP 500 mg	75 g
Tetrax R 500		150 g
Acizol 20	N, N-methylene-bi2-amino-5-thiol-1,3,4-	300 g
Acizol 20	Thiadiazole	600 g
Thiasol 20	N, N-methylene-bi2-amino-5-thiol-1,3,4-	1850 g
Thiasol 20	Thiadiazole	3700 g
Krosin AG 10 SP	Streptomycin Sulphate + Tetracycline	200 g
Krosin AG 10 SP	hydrochloride (9:1) SP	400 g
Dibacteria 10 SP	Streptomycin Sulphate + Tetracycline	200 g
Dibacteria 10 SP	hydrochloride	400 g
Sunbenchlop 37 WP	Benalaxyl 4% + Copper oxychloride 33%	4000 g
Sunbenchlop 37 WP		8000 g
Diseased control (no chemicals but inoculated)	-	

Plants inoculated with BB inoculum without spraying chemicals were used as control to compare the efficacy of tested chemicals.

Efficacy of eight nonregistered and newly imported chemicals recommended as bacteriocides by the manufacturer (Table 1) were tested against bacterial blight pathogen *(Xanthomonas oryzae* pv. *oryzae)* under laboratory conditions (*in-vitro*) and against BB disease in net house and field under inoculated conditions. Recommended dose as well as doubling of the recommended dose were tested.

In vitro screening of the tested chemicals

In vitro screening of the selected chemicals was performed following paper disc diffusion method (Panda *et. al.*, 2009). Fresh culture of the test bacteria BXO9 was seeded over the Peptone Sucrose Agar (PSA) plate with a sterile swab.

Sterile filter paper discs with diameter of 6 mm were soaked into 50 µl specific chemicals (1:1 W/V or V/V) followed by air drying to evaporate the solvent in the laminar flow. Pure streptomycin in soaked disc (10 µg/disc), Himedia Laboratories Pvt. Ltd., Mumbai, India was used for comparison of efficacy of the bactericides against BB pathogen. The air dried discs and control discs were placed on the seeded plates at equidistance and were incubated at 37^0C for 72 h. After the incubation period, a clear zone which is known as inhibition zone around the discs was observed which indicated a positive antibacterial activity of the chemicals. The clear zone were formed around each disc was measured. The mean of the inhibition zone ± SE was taken for evaluating the antibacterial activity of chemicals.

Net house trial

Evaluation of chemicals in net house was performed by artificial inoculation of rice plants with a virulent isolate BXO9. Rice plants of a susceptible variety 'Purbachi' were grown in pots. The pots were placed in the net house and inoculated with freshly prepared bacterial suspension (108 to 1010 CFU/ml). Total seventeen treatments including sixteen chemicals and one disease control were maintained during experimentation (Table 1). The chemical were suspended in water and applied as preventive and curative methods. For preventive, all the chemicals were applied before inoculation and in curative, all the chemicals were sprayed after inoculation with bacterial suspension at 108 to 1010 CFU/ml. The spray schedule were 4 and 14 days before inoculation for preventive and 4 and 14 days after inoculation for curative. Two factors bacterial experiment in randomized complete block design (RCBD) was followed with four replications; as a result 136 pots were maintained in the experiment. The mean were compared using Tukey's Honest Significant differences (HSD) test.

Field trial

Field evaluations were done following the same procedure of net house trial maintaining three replications. In total 102 unit plots (1m X 1m) of two factors RCBD maintained during experimentation.

Inoculum preparation, Inoculation and data collection

The BB isolate BXO9 was cultured on PSA slants for 72 hours at 30ºC. Inoculum was prepared by mixing the cultured bacteria with 10 ml sterile distilled water in a slant. Before inoculation the concentration of the bacterial suspension was adjusted to 108 to 1010 CFU/ml using sterile distilled water. The plants at maximum tillering stage were inoculated by leaf clipping method (Kauffman *et. al.,* 1973) in this experiment. Data of lesion length, infected leaf area (ILA) and disease score (DS) were collected according to Standard Evaluation System (IRRI, 2013) at 21 days after inoculation. The disease index (DI) was measured using the following formula:

$$DI = 100 \text{ X } \frac{\text{Sum of the all disease rating}}{\text{No. of leaves scored X 9}}$$

(Singh *et. al.,* 1980). Randomly 20 clipped leaves were selected from each treatment for data collection and scored on a 0-9 scale following IRRI (2013) and finally the scores were converted to DI.

Results

***In vitro* screening of chemicals against bacterial blight pathogen**

Among the tested chemicals, streptomycin based bacteriocides Krosin AG 10SP and Sunbenchlop 37WP showed the maximum inhibition zones of 54.1 ± 0.5 mm and 53.1 ± 0.4 mm, respectively, where as control plate did not show any inhibition zone (Fig. 1). To confirm the efficacy of streptomycin bactericide against *X. oryzae pv. oryzae*, laboratory grade streptomycin (10µg/disc) was also used for the comparison. Results confirmed the potentiality of streptomycin in inhibiting BB pathogen that produced maximum inhibition zone 59.3 ± 0.8 mm under laboratory condition (Fig. 1).

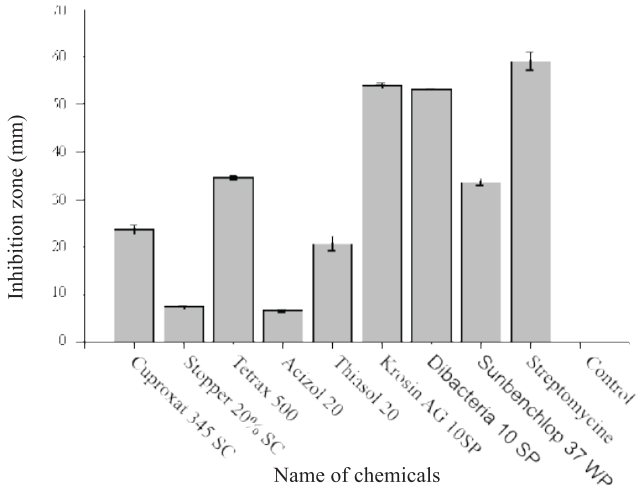

Fig. 1. Antibacterial activity of selected chemicals against *X. oryzae pv. oryzae* by disc diffusion method. Laboratory grade streptomycin was used for comparative antibacterial activity between streptomycin based chemicals and laboratory grade streptomycin against BB pathogen by disc diffusion method. Zone of inhibition of mean ± SE in mm; Zone of inhibition including 6 mm disc.

Effect of chemicals on BB development in nethouse

The lowest lesion length (9.7 cm), infected leaf area (9.3%) and disease index (30.56) were found when applied either single or double doses of Thiadiazole based chemical Thiasol 20, whereas in control plot these parameters showed the maximum values of 32.46 cm, 85.17% and 100, respectively (Table 2). Other Thiadiazole based chemicals such as BLB Stopper 20% and Acizol 20 provided second lowest values of those parameters. Though Streptomycine based chemicals (Krosin AG 10 SP, Krosin AG 10 SP, Dibacteria 10 SP and Dibacteria 10 SP) perform best in *in vitro* condition, there was no such type of performance in BLB suppression under nethouse condition.

Table 2. Effect of chemicals on bacterial leaf blight of rice under nethouse condition

Treatments	Lesion length (cm)	Leaf area infected (%)	Disease index
Cuproxat 345 SC	24.11	34.08	72.22
Cuproxat 345 SC	21.74	43.38	80.56
BLB Stopper 20% SC	16.46	27.88	58.33
BLB Stopper 20% SC	14.02	18.45	50.00
Tetrax R 500	19.70	37.87	72.22
Tetrax R 500	21.25	40.76	75.00
Acizol 20	18.86	26.90	52.78
Acizol 20	16.22	15.99	41.67
Thiasol 20	9.71	10.05	30.56
Thiasol 20	11.98	9.30	36.11
Krosin AG 10 SP	18.60	32.79	69.45
Krosin AG 10 SP	16.48	22.05	55.56
Dibacteria 10 SP	22.07	31.80	77.78
Dibacteria 10 SP	19.38	26.33	58.33
Sunbenchlop 37 WP	19.43	28.66	63.89
Sunbenchlop 37 WP	22.26	37.92	75.00
Control	32.46	85.17	100
LSD (*P=0.05*)	5.45	14.46	16.67

Effect of chemicals on BB development in the field

Bacterial Blight disease parameters such as disease incidence, disease severity and disease index in field are presented in (Table 3). Among the disease parameters, lowest disease severity (26.24%) and disease index (62.97) were found when applied Thiasol 20 as preventive followed by Krosin AG 10SP also as preventive, where as in control plot the maximum values were recorded 67.94% and 100, respectively of these parameters. The interaction results between chemicals and application times depicted that in most of the cases, lowest disease parameters were always obtained from preventive treatment for each of the chemicals. Disease severity was decreased to some extent after chemical application but there was no significant difference between control plot and chemical treated plots.

Effect of chemicals on yield and yield components

The yield contributing characters such as filled grain/panicle, grain sterility, thousand grain weights and grain yield of rice variety Purbachi were not influenced consistently among the treatments in field experiments. Though Thiasol treated plot provided maximum yield (2.51 t/ha), but grain sterility percentages were also high under that treatment (Table 4).

Table 3. Effect of chemicals on bacterial blight of rice under field condition

Treatments	Disease incidence		Disease severity		Disease index	
	Preventive	Curative	Preventive	Curative	Preventive	Curative
Cuproxat 345 SC	100	100	43.74	46.75	77.78 bc	77.78 bc
Cuproxat 345 SC	100	100	38.74	41.40	77.78 bc	77.78 bc
BLB Stopper 20% SC	100	100	35.27	37.11	77.78 bc	77.78 bc
BLB Stopper 20% SC	100	100	29.57	30.61	77.78 bc	77.78 bc
Tetrax R 500	100	100	51.24	55.78	92.59 ab	100.00 a
Tetrax R 500	100	100	44.57	45.71	77.78 bc	77.78 bc
Acizol 20	100	100	46.24	47.27	77.78 bc	77.78 bc
Acizol 20	100	100	32.07	34.81	77.78 bc	77.78 bc
Thiasol 20	100	100	26.24	27.27	62.97 c	70.37 b
Thiasol 20	100	100	35.27	37.11	77.78 bc	77.78 bc
Krosin AG 10 SP	100	100	26.76	28.78	70.37 c	77.78 bc
Krosin AG 10 SP	100	100	41.24	45.78	77.78 bc	77.78 bc
Dibacteria 10 SP	100	100	36.76	38.78	77.78 bc	77.78 bc
Dibacteria 10 SP	100	100	39.57	40.61	77.78 bc	77.78 bc
Sunbenchlop 37 WP	100	100	54.57	55.61	100.00 a	100.00 a
Sunbenchlop 37 WP	100	100	40.40	42.69	77.78 bc	77.78 bc
Control	100	100	97.94	67.94	100.0 a	100.00 a
HSD (*P=0.05*)	NS	NS	NS	NS	15.79	9.52

HSD: Tukey's Honest Significant Difference; NS: Not significant
Means with the same letter are not significantly different

Table 4. Interaction effect of chemicals and its application time on yield and yield contributing characters of rice under field condition

Treatments	Yield and yield contributing characters			
	Filled grain/ panicle (No.)	Grain sterility (%)	1000-grain weight (gm)	Yield (t/ha)
Cuproxat 345 SC @ 1 L ha^{-1} X Preventive	90.47	47.80	11.17	1.70
Cuproxat 345 SC @ 1 L ha^{-1} X Curative	76.33	38.63	10.30	1.64
Cuproxat 345 SC @ 2 L ha^{-1} X Preventive	84.93	45.53	10.77	1.34
Cuproxat 345 SC @ 2 L ha^{-1} X Curative	83.33	44.87	10.87	1.34
BLB Stopper 20% SC @ 1200 ml ha^{-1} X Preventive	68.27	45.50	10.13	1.51
BLB Stopper 20% SC @ 1200 ml ha^{-1} X Curative	99.40	47.57	11.57	1.92
BLB Stopper 20% SC @ 2400 ml ha^{-1} X Preventive	69.53	43.57	10.37	1.84
BLB Stopper 20% SC @ 2400 ml ha^{-1} X Curative	66.73	33.73	10.01	1.80
Tetrax^{R} 500 @ 3 cap/10L X Preventive	99.9	34.83	12.40	1.50
Tetrax^{R} 500 @ 3 cap/10L X Curative	74.53	32.30	10.47	1.75
Tetrax^{R} 500 @ 6 cap/10L X Preventive	74.53	35.60	10.80	1.68
Tetrax^{R} 500 @ 6 cap/10L X Curative	86.33	43.01	10.93	1.65
Acizol 20 @ 300 g ha^{-1} X Preventive	89.13	47.27	10.23	1.87
Acizol 20 @ 300 g ha^{-1} X Curative	90.33	38.87	10.77	1.70
Acizol 20 @ 600 g ha^{-1} X Preventive	78.13	44.90	10.87	2.01
Acizol 20 @ 600 g ha^{-1} X Curative	86.27	45.73	10.67	2.18
Thiasol 20 @ 1.85 kg ha^{-1} X Preventive	82.01	34.53	11.07	2.25
Thiasol 20 @ 1.85 kg ha^{-1} X Curative	103.0	34.33	11.03	2.51
Thiasol 20 @ 3.70 kg ha^{-1} X Preventive	87.13	54.10	11.07	1.97
Thiasol 20 @ 3.70 kg ha^{-1} X Curative	98.53	33.57	12.17	2.02
Krosin AG 10 SP @ 200 g ha^{-1} X Preventive	85.20	35.70	10.13	1.91
Krosin AG 10 SP @ 200 g ha^{-1} X Curative	68.73	34.33	10.01	2.04
Krosin AG 10 SP @ 400 g ha^{-1} X Preventive	90.01	44.20	10.87	1.71
Krosin AG 10 SP @ 400 g ha^{-1} X Curative	92.60	46.67	10.60	1.54
Dibacteria 10 SP @ 200 g ha^{-1} X Preventive	95.40	37.80	11.57	1.74
Dibacteria 10 SP @ 200 g ha^{-1} X Curative	70.80	42.27	10.97	1.64
Dibacteria 10 SP @ 400 g ha^{-1} X Preventive	99.53	36.37	10.77	1.94
Dibacteria 10 SP @ 400 g ha^{-1} X Curative	59.87	45.73	10.53	1.64
Sunbenchlop 37 WP @ 4 kg ha^{-1} X Preventive	69.80	41.93	10.30	1.18
Sunbenchlop 37 WP @ 4 kg ha^{-1} X Curative	84.60	40.73	10.43	2.08
Sunbenchlop 37 WP @ 8 kg ha^{-1} X Preventive	85.67	44.97	10.67	1.78
Sunbenchlop 37 WP @ 8 kg ha^{-1} X Curative	78.13	35.90	10.81	2.01
Diseased control X Preventive	99.5	35.60	12.30	1.74
Diseased control X Curative	100.3	32.80	11.13	1.94
LSD (*P=0.05*)	33.90	8.05	3.29	0.74

Note: Grain moisture was adjusted at 14%; $1m^2$ grain yield was converted to t/ha.

Discussion

In the present study, streptomycin based bactericides Krosin AG 10SP and Dibacteria 10SP inhibited bacterial growth *in vitro*. It suggested that there is a possibility of streptomycin based bactericides to inhibit *Xanthomonas oryzae* pv. *Oryzae.* Similar result was obtained when pure streptomycin was tested as a standard against the same pathogen under the same condition. These results are in agreement with the findings of Levaditi and Chaigneau-Erhard (1951), Durgapal (1983), Rakesh (1994) and Mary *et. al.,* (2001). They found strong inhibitory potentiality of streptomycin against BB pathogen under both laboratory and field conditions.

In both net house and field experimentation conditions, Thiosol 20 provided the best performance in controlling BB up to 21 days after inoculation. The name of Thiosol indicates the presence or reach of sulfur (S) in that chemical. Due to the presence of S in bactericides, it helped to turn the leaf colour more greenish quickly. That is why, leaf of Thiosol 20 sprayed plant showed more greenish and showed low infected leaf area initially (up to 21 days of bacterial inoculation).

Between two chemical spray times, preventive application provided better result than curative under net house, but in field condition no significant differences were found between the application times of chemical spray. Now-a-days, most of the pesticide companies are added elemental S in their product to show quick effect of their chemicals by changing the rice leaf color. That is why, during bacterial inoculation, most of the plants of preventive application were comparatively more green than curative except control plot. Due to the deep color of the leaf, initial progress of the disease was low in preventive treatment.

Though initially some of the chemicals and their interaction with application time gave good results in controlling BB under both nethouse and field conditions, but finally none of the chemicals were found effective against bacterial blight (BB) under net house or field condition. Even though, positive effect of the tested chemicals on yield and yield contributing characters in field trial were also inconsistent. Therefore, further study is necessary to search for effective chemicals against BB disease of rice in Bangladesh.

Conclusion

Among eight non registered chemicals tested in the present study Krosin AG 10SP and Dibacteria 10SP show strong antibacterial activity *in vitro* but all of them are ineffective against bacterial blight (BLB) under net house as well as field conditions.

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EFFICACY OF TRICHODERMA TO CONTROL ROOT ROT *(FUSARIUM SOLANI)* OF LENTIL AND SHEATH BLIGHT *(RHIZOCTONIA SOLANI)* OF RICE

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Abstract

A series of experiments were carried out in the laboratory and experimental field of Bangladesh Institute of Nuclear Agriculture, Mymensingh to assess the efficacy of some isolates of *Trichoderma harzianum* Rifai in controlling root rot (*Fusarium solani*) of lentil and sheath blight (*Rhizoctonia solani* Kuhn) of rice. Nine isolates of *Trichoderma* were tested against *F. solani* and *R. solani* following dual culture method. The highest mycelium growth inhibition against *F. solani* (99.10%) and *R. solani* (98.00%) were obtained with the isolate TRD-10 followed by TRD-8. In a field experiment, efficacy of TRD-10 and TRD-8 were tested against root rot *(F. solani)* of lentil. The isolate TRD-10 was more effective than TRD-8 to reduce disease incidence and to increase seed germination, plant stand, plant growth and grain yield. Soil treatment with TRD-10 reduced disease incidence by 68.40%, and increased seed germination by 53.0 and plant stand by 45.9% and grain yield by 243.8% over control. In another field experiment, effect of application of TRD-10 on incidence and severity of sheath blight *(R. solani)* and grain yield of rice was evaluated under natural condition in three locations, Ishurdi, Magura and Mymensingh. Incidence of sheath blight was 99.4, 84.0 and 99.7% under control in three locations, respectively. Due to pre-transplanting seedling treatment, foliar spray, pre-planting soil treatment and soil treatment + spray with the isolate TDR-10, the incidence was reduced to 20.7-67.4, 18.5-45.2 and 21.9-54.8%, respectively. The yield was increased to 1.01-1.40, 0.57-1.02 and 0.38-0.81 kgm^{-2} due to application of six different treatments in three locations, respectively. The most effective treatment was soil treatment + 3 times spray followed by only pre-planting soil application and pre-planting seedling treatment. Considering the above findings, isolate TRD-10 of *T. harzianum* may be recommended to use a biological management tactic against root rot of lentil and sheath blight of rice. However, further study is necessary to determine benefit cost ratio.

Introduction

Lentil (*Lens culinaris* Medik.) is an important pulse crop in Bangladesh. It provides a good source of protein (30 to 35%), but are limited in the amino acids methionine and cystine (Kandel and Ashley, 2013) and 48% carbohydrate (Feedipedia, 2012). Out of eight kinds of pulses, lentil, being the rich source of vegetable protein, is a common item in the daily diet of the people of Bangladesh (Bakr *et. al.*, 1997).

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The yield of lentil (1.5 t/ha; BARI, 2010) is much lower in Bangladesh compared to other pulse growing countries like Syria, Turkey, Canada, USA and Ethiopia. There are various causes associated with low yield of lentil in Bangladesh, where diseases are considered as the major constraints resulting about 30-40% yield loss (Begum, 2003). Of the major diseases root rot (*Fusarium solani*) of lentil is an important soil borne disease, which may cause up to100% seedling mortality in the field under monoculture and favorable weather condition (Begum, 2003).

Rice (*Oryza sativa* L.) is the main staple food for 160 million people of Bangladesh. More than 75% of cultivable land is being used for rice cultivation. A total of 31 rice diseases are known to occur in Bangladesh among which sheath blight *(R. solani)* is the most important and devastating one limiting rice yield (Miah *et. al.*, 1985). The disease can result in a loss of crop production up to 45% under favorable condition (Kumar *et. al.*, 2009) and in Bangladesh causes 14-31% grain yield loss (Shajahan *et. al.*, 1986).

Both *F. solani* and *R. solani* are soil borne fungi. As there is no effective fungicide or resistant variety for the management of these soil-borne diseases farmers cannot maintain optimum plant population in the field and the yield of crop is drastically reduced. The potential of the antagonistic microorganisms for reducing crop damage are known (Lewis and Larkin, 1997). The efficacy of fungal antagonists (*Trichoderma viride, T. harzianum* and *Gliocladium virens*) are found effective as seed treatment agents the root rot pathogen (Dubey and Patel, 2002). In biological control, Trichoderma has an antagonistic character against many soil-borne fungi such as *R. solani*, Sclerotium rolfsii and *F. solani* (Chet and Inbar, 1994). This antagonist increased the percentage of seedling emergence, plant height, fresh and dry weight, number of nodules, total N and total protein content of the plants. *Trichoderma harzianum* produces IAA and certain vitamins which seemed to play a role in yield increase (Ychia *et. al*., 1994). Biological control can be safer for human, the crop and the environment and it also allows a reduction in the use of chemical pesticides. So, biological management of soil-borne diseases increasingly gaining stature as a possible practical and safe approach (Patel and Anahosur, 2001). Considering the above facts, the present study was undertaken to assess the efficacy of some isolates of *Trichoderma* spp. to control the root rot of lentil and sheath blight of rice.

Materials and Methods

Diseased plant samples of root rot and sheath blight and field soils were collected Table 2. Effect of soil treatments with *T. harzianum* on root rot incidence, seed germination, plant stand, growth parameters and yield of lentil inoculated with *F. solani* from different cropping area of Bangladesh. Nine isolates of *T. harzianum* were isolated from pulse and rice growing fields. *Fusarium* and *R. solani* were isolated from diseased plant samples. Isolation, identification, purification and preservation were done following the method as used by Rifai (1969), Singh (1982), Kamal (1992) and Begum (2003).

In vitro evaluation of *Trichoderma* isolates against *F. solani* and *R. solani*

Antagonistic effect of different isolates of *T. harzianum* on invitro mycelium growth of *F. solani* and *R. solani* were performed following dual culture technique. Two experiments were carried out in the laboratory of Plant pathology Division, Bangladesh Institute of Nuclear Agriculture, Mymensingh, Bangladesh during 2010 to 2011 following

completely randomized design (CRD) with five replications. PDA plates were inoculated with a 6 mm diameter blocks cut from 7 days old PDA culture of *Trichoderma*. The blocks were placed upside down on PDA as suggested by Sultana (1999). The distance between the inoculum blocks in plates were 7 cm. Control plates contained pathogens only at the center of the PDA plates. The plates were then incubated at 28 ± 1^0C and observation on growth inhibition was made up to 10 days for *F. solani* and 6 days for *R. solani.* The percent inhibition was calculated following the formula as suggested by Sundar *et. al*., (1995).

$$\text{Inhibition (\%)} = \frac{X - Y}{X} \times 100$$

Where,

X = Mycelial growth of pathogen without antagonist

Y = Mycelial growth of pathogen along with the antagonist Field evaluation of *Trichoderma* isolates against root rot

Field evaluation of *Trichoderma* isolate for controlling root rot of lentil

Two isolates of *Trichoderma* sp. (TRD-8 and TRD-10) that showed better performance to reduce invitro growth of *F. solani* in dual culture test were evaluated in the field soil naturally infested with *F. solani*. The land was prepared by four ploughings and cross ploughings. The land was then left exposed to natural weathering for ten days and final ploughing and proper leveling were done before sowing. The seed (Binamasur 3) rate was 25 Kg ha-1. Number of seeds for each unit plot was counted before sowing. The experiment was laid out in a randomized complete block design with three replications. The unit plot size was 2m x 2m and there were 6 rows (30 cm a part) in each plot. There were three treatments in the experiment: (i) *T. harzianum* (TRD-10), (ii) *T. harzianum* (TRD-8) and (iii) control (*F. solani* alone).

The isolates of *T. harzianum* were grown on chickpea bran following the method of Kashem *et. al.,* (2005). The chickpea bran was soaked in water at the ratio of 3:4 (w/v). Water soaked chickpea bran (500 g) was taken in a beaker (2L) and autoclaved at 121^0C for 30 minutes at 1.1 kg/cm2. The sterilized chickpea bran was inoculated with 20 blocks (6 mm diameter) of *T. harzianum* previously grown on PDA and incubated at room temperature (28^0C) for 15 days. The inoculum of *Trichoderma* was applied in the field in rows @ 10gm-1at the time of seed sowing (Raguchander *et. al.*, 1997).

Soil was moistened when necessary. Weeding was done three times during the crop growing period. No chemical pesticide was applied for controlling pests and diseases. Data on seedling emergence, root rot disease incidence, plant growth and yield attributes were recorded at different stages of crop growth.

Field evaluation of *Trichoderma* isolate for controlling sheath blight of rice

Trichoderma harzianum (TRD-10) that showed better performance to reduce *invitro* mycelium growth of *R. solani* in dual culture test was selected to evaluate its activity in the field against sheath blight of rice (TN-1) at Ishurdi, Magura and Mymensingh during boro season of 2013. The experiments had six treatments viz. seedling treatment with TRD-10, spray with TRD-10 at 20, 40 and 60 DAT, soil treatment with TRD-10, soil treatment + 3 times spray with TRD-10 (20, 40, 60 DAT) and control.

The experiments were conducted in a randomized complete block design with three replications. Replication to replication distance was 1m. The unit plots size, row to row and plant to plant distance were 2mx2m, 30cm and 15cm, respectively. The seedlings were transplanted on late February to early March 2013. Data on disease severity were taken at booting and ripening stages following 0-9 disease scale (IRRI, 2002).

Results and Discussion

In vitro* evaluation of *Trichoderma* isolates against *F. solani* and *R. solani

The antagonistic potentiality of nine isolates of *T. harzianum* showed wide variations in growth inhibition of *F. solani* that varied 2.10-13.77, 22.08-76.35, 42.87-92.13 and 61.32-99.10% at 3, 5, 7 and 10 days after inoculation (DAI), respectively. The inhibition was significant under all isolates at 5 and 7 DAI compared to control. The isolate TRD-10 showed the highest inhibition followed by TRD-8 (94.91%) and TRI-1 (94.32%) (Table1). In a dual culture study *Trichoderma* spp. were observed as highly inhibitory to *F. solani* (Karunanithi and Usman, 1999). Ngueko and Xu (2002) reported that *T. harzianum* reduced the mycelial growth of *F. solani* by 52-87%. The antagonism between *T. viride* and *F. solani* was observed in another in vitro test where inhibition of 97% was recorded (Tian *et. al*., 2001). Vyas and Mathur (2002) reported the effective inhibition of growth and sporulation of *F. oxysporum* with *Trichoderma* spp.

For *R. solani* the growth inhibition by eight isolates of *T. harzianum* varied 3.0-9.95, 36.33-72.55 and 69.41-98.00% at 3, 4 and 6 DAI, respectively. The inhibition was significant under all isolates at all stages of data collection compared to control. The isolate TRD-10 showed the highest inhibition followed by TRD-8 (96.32%) (Table1). Similar findings was also reported by Neimi *et. al.,* (2010). They found in an in vitro antagonism test between *R. solani* and *T. harzianum* that 83.3% isolates of total 78 isolates of *Trichoderma* were antagonistic to *R. solani.*

Several mechanisms may explain the inhibition activity of *F. solani* and *R. solani* by *Trichoderma* isolates. Hyperparasitism and volatile metabolites might be involved in the inhibition of pathogens by *Trichoderma*. Cell wall degrading enzymes such as chitinase, glucanase and proteases are thought to be closely related to the mycoparasitsm of *Trichoderma* strains (Elad and Freeman, 1996; Harman *et. al*., 2006) where as Claydon *et. al*., (1987) observed the inhibitory effect of volatile substances such as alkyl pyrons to *Trichoderma* isolates.

Field evaluation of *Trichoderma* isolates for controlling root rot of lentil

The maximum of 40.20% root rot incidence was recorded under control. Both isolates of *T. harzianum*, TRD-10 and TRD-8 reduced the root rot incidence of lentil by 68.40 and 51.50%, respectively over control. The reduction was significant ($P \geq 0.05$) compared to control.

In lentil, root rot incidence, seed germination and the survival of plants were significantly influenced by the application of *T. harzianum* in to soil (Table 2). The lowest root rot incidence (12.7%) was recorded in plots where the isolate TRD-10 was incorporated

to the soil. The lowest seed germination of 54.3% and plant stand of 59.8% were found under untreated control. The germination increased by 53.0 and 32.9% and plant stand increased y 45.9 and 34.6% over control due to application of TRD-10 and TRD-8, respectively. Application of isolates TRD-10 increased fresh shoot weight root, weight, number of pod, weight of pod and grain yield by 202.87, 172.84, 113.9, 289.4 and 243.8%, respectively. TRD-8 gave increase in fresh shoot weight, root weight, weight of pod and grain yield by 149.76, 119.32, 184.0 and 158.4% over control, respectively. The increase in germination, plant stand, growth parameters and yield attributes were significant compared to control (Table 2).

The results of the present experiment was in agreement with other investigators. The field where the experiment was conducted had been utilized for lentil cultivation for the last three consecutive years and the occurrence of root rot was a normal phenomenon every year. Mukhopadhyay (1989) reported that the biological treatment with *T. harzianum* in lentil gave an excellent protection against a wide range of pathogens including *F. solani. Trichoderma* spp. were successfully used for controlling root rot diseases of lentil (Heemert *et. al*., 2000; Vyas and Mathur, 2002; Prasad *et. al*., 2002). In the present experiment, 53% higher germination of lentil was recorded due to application of *T. harzianum* (TRD-10) compared to control. Inoculation with *Trichoderma* isolates reduced the incidence of root rot and increased seed germination in lentil (Raju *et. al.*, 1999; Vyas and Mathur, 2002). Prasad *et. al.* (2002) found significantly higher seed yield when field soil was treated with *T. harzianum* and *T. viride* against root rot of chickpea. *Trichoderma* strains produce a large number of chemicals that strongly influence plant nutrient solubilization and absorption by roots and thus enhance the growth of plants resulting in increased yield (Singh, 2006). *Trichoderma harzianum* produces Indole Acetic Acid (IAA) and certain vitamins which seem to play a role in yield increase (Ychia *et. al*., 1994).

Table 1. Effect of different isolates of *T. harzianum* on in vitro growth inhibition of *F. solani* and *R. solani* in dual culture technique

Trichoderma Isolates	*In vitro* growth inhibition of *F. solani* (%)				*In vitro* growth inhibition of *R. solani* (%)		
	3 DAI	5 DAI	7 DAI	10 DAI	3 DAI	4 DAI	6 DAI
TRD-3	5.82	53.08	65.64	82.65	9.04	60.36	84.76
TRD-10	13.77	76.35	92.13	99.10	8.87	71.25	98.00
TRD-8	10.22	68.55	85.98	94.91	7.04	72.55	96.32
TRD-21	5.17	28.98	47.87	61.32	3.50	36.33	69.41
TRD-15	7.14	72.15	83.00	92.61	9.95	64.65	88.20
TRS-1	2.10	47.28	54.48	64.37	7.47	54.82	85.60
TRI-1	12.50	65.25	83.42	94.32	9.77	68.70	87.55
TLN-7	5.64	27.77	48.19	62.98	3.04	39.31	78.11
TLN-27	5.19	22.08	48.14	62.43	3.00	42.80	77.38
Control	0.0	0.0	0.0	0.0	0.0	0.0	0.0
$LSD_{0.05}$	9.20	8.55	6.33	5.44	2.04	4.33	5.61

Table 2. Effect of soil treatments with *T. harzianum* on root rot incidence, seed germination, plant stand, growth parameters and yield of lentil inoculated with *F. solani*

Trichoderma harzianum isolates	Root rot incidence (%)	Seed germination (%)	Plant stand (%)	Fresh shoot weight (gm^{-2})	Fresh root weight (gm^{-2})	Number of pod m^{-2}	Fresh weight of pod (gm^{-2})	Dry grain yield ($kgha^{-1}$)
TRD-10	12.70 (-68.40)	83.1 (+53.0)	87.3 (+45.9)	633.00 (+202.87)	24.01 (+172.84)	7523.0 (+113.9)	366.0 (+289.4)	3507.0 (+243.8)
TRD-8	19.5 (-51.50)	72.2 (+32.9)	80.5 (+34.6)	522.00 (+149.76)	19.30 (+119.32)	1420.0 (-59.6)	267.0 (+184.0)	2636.0 (158,4)
Control	40.20	54.3	59.8	209.00	8.80.00	3517.0	94.0	1020.0
$LSD_{0.05}$	5.70	15.60	12.5	25.30	402.00	27.8	21.7	70.5

Data in parenthesis indicate percent increase (+) or decrease (-) over control. Data represent the means of three replications root rot incidence (%), seed germination (%) and plant stand (%) 68.40, 53.0 and 45.9.

Field evaluation of *Trichoderma* isolate for controlling sheath blight of rice

Incidence of sheath blight of rice was 99.4, 84.0 and 99.7% at Ishurdi Magura and Mymensingh, respectively under control. Due to pre-transplanting seedling treatment, foliar spray, pre-planting soil treatment and soil treatment + spray with *T. harzianum* isolate TDR-10, the incidence was reduced to 20.7-67.4, 18.5-45.2 and 21.9-54.8%, respectively in those three locations. The reduction was significant compared to control. The most effective treatment was soil treatment + 3 times spray followed by only pre-planting soil application and pre-planting seedling treatment with TRD-10 (Table 3).

The highest sheath blight severity of 7% was recorded from control plots in all three locations. Due to application of six treatments the severity was reduced to 1-3, 1-5 and 3-5% in Ishurdi, Magura and Mymensingh, respectively. The reduction was significant compared to control. Under control, the grain yield was 0.88, 0.65 and 0.33 kgm^{-2} in Ishurdi, Magura and Mymensingh and the yield was increased to 1.01-1.40, 0.57-1.02 and 0.38-0.81 kgm^{-2} due to application of six different treatments in three locations, respectively. The increase was significant under all treatments in Ishurdi but under only soil application alone and soil application + 3 times spray in other two locations compared to control (Table 3).

The genus *Trichoderma* is known for its antagonistic activity against several plant pathogens, including *R. solani* (Harman, 2006). In present investigation, however, soil application of *T. harzianum* was found to be better than seed treatment to reduce disease incidence and severity and to increase grain yield. The results are in conformity with those of Elad and Freeman (1996). Mostafa Kamal and Shahjahan (1995) isolated 360 *Trichoderma* strains from rice fields in different locations of Bangladesh and reported that *T. harzianum* was prevalent in the rice fields. Therefore, in present study when *T. harzianum* was applied in the soil it might be able to survive and multiply well in its suitable habitat and show its antagonistic activity against plant pathogenic fungi.

Table 3. Effect of TRD-10 isolate of *Trichoderma harzianum* at different type of application on sheath blight incidence, severity and yield of rice inoculated with *Rhizoctonia solani* in three locations

Treatments	Sheath bight incidence (%)			Ishurdi		Magura		Mymensingh	
	Ishurdi	Magura	Mymensingh	Disease severity %	Yield (kgm^{-2})	Disease severity %	Yield (kgm^{-2})	Disease severity %	Yield (kgm^{-2})
Seedling treatment	57.9	37.3	54.8	3	1.03	5	0.78	5	0.40
Foliar spray at 20 DAT	59.9	39.4	54.3	3	1.01	5	0.66	5	0.38
Foliar spray at 40 DAT	67.4	45.2	51.9	3	1.03	5	0.76	5	0.40
Foliar spray at 60 DAT	62.6	44.9	45.2	3	1.04	5	0.57	5	0.40
Pre-planting Soil treatment	35.2	25.0	34.4	3	1.14	3	0.98	3	0.44
Soil treatment + 3 times spray (20, 40, 60 DAT)	20.7	18.5	21.5	1	1.40	1	1.02	3	0.81
Control	99.4	84.0	99.7	7	0.80	7	0.65	7	0.33
$LSD_{0.05}$	7.8	5.3	7.3	1.5	0.40	1.7	0.27	1.4	0.31

DAT = Days after transplanting,

Data represent the means of three replications

Conclusion

Findings of the present study reveal that among nine isolates of *T. harzianum*, TRD-10 and TRD-8 are most effective to inhibit in vitro mycelium growth of *F. solani* and *R. solani.* These two isolates are also effective to reduce incidence of root rot *(F. solani)* and seedling mortality and to increase grain yield of lentil under field conditions. The most effective isolate is TRD-10. The isolate is also very effective to control sheath blight *(R. solani)* of rice and to improve plant growth as well as grain yield of rice. Application of TRD-10 for pre-planting soil treatment alone or soil treatment + 3 times sprays at 20, 40 and 6 days after transplanting may be recommended as a biological management tactic of root rot of lentil and sheath blight of rice. However, estimation of benefit cost ratio is required before final recommendation.

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meters (Saari and Prescott, 1975). The fungus reduces the yield of wheat as a result thinner stand with lower number, size and kernel weight. The effect of pathogen on crop yield and quality may vary considerably from year to year relating to changes in the environmental factors Kumar *et. al.*, (2009) stated that spot blotch caused by *Bipolaris sorokiniana* is a destructive disease of wheat in warm and humid wheat growing regions of the world. The losses of wheat yield due to diseases caused by it are variable depending upon the weather conditions. Mehta (1985) reported that under favorable weather the pathogen could result the losses in wheat yield between 30-80%, in some cases the losses could up to 100%. Dubin and Ginkel (1991) reported that the severity of the disease was more in the warmer areas than in Temperate Zone. The severity of bipolaris leaf blight as been increased several days later depending on the resistance level of the genotype. The disease severity depends on the sensitivity of the cost as well as on the climatic factors, specially temperature. Leaf blotch caused by Bipolaris could be maximum at any growing period of the plants if the environmental factors, pathogen and cost could be placed at optimum condition (Hossain and Azad, 1992). Hossain and Azad (1994) found that wheat plants are more vulnerable to *H. sativum* at different growth stages depending on the environmental condition and found that at severe infection at flag leaf stage resulted 7-100% reduction in formation of grains per ear. Zhimin *et. al.,* (1997) in a study that the Bipolaris sorokiniana is the most important accounting for more than 80% of fungal strains collected. Annual yield losses due to Bipolaris sorokiniana are estimated at 10-15%. Greatest damage occurs during the flowering to milk stages of wheat plants. Disease severity is closely related to temperature and humidity. So the present findings in agreement with the findings of Saari and Wilcoxon, 1974, Kumar *et. al.*, 2009 and Mehta 1985 i.e. environmental factors has a great influence on the development of leaf blight disease of wheat.

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