SURVEY ON RICE BLAST IN SOME SELECTED AREA OF BANGLADESH AND *IN-VITRO* EVALUATION OF FUNGICIDES AGAINST *PYRICULARIA ORYZAE*

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ABSTRACT

Rayhanul, M. I., Aminuzzaman, F. M., Chowdhury, M. S. M., Laila, L. and Ahmed, M. 2019. Survey on rice blast in some selected area of Bangladesh and *in vitro* evaluation of fungicides against *Pyricularia oryzae*. Bangladesh J. Plant Pathol. 35(1&2):59-64

Survey was conducted on rice blast in 110 rice field of 12 upazillas under 5 districts of Bangladesh namely Mymensingh, Kishoreganj, Barishal, Naogaon and Cumilla. Among the survey areas Muktagachha, Mymensingh was found as the highest rice blast disease infected area and Bakerganj, Barishal was found as the lowest in Boro season 2017-2018. *Pyricularia oryzae*(*Po*)was isolated from infected leaf and panicle and identified based on cultural characteristics and conidia morphology. Mycelial growth of four *Po* isolates varied significantly with fair to excellent sporulation ability. Eight fungicides namely Trooper 75WP, Seltima 100CS, Nativo 75 WP, Amistar Top 250 SC, Azonil 56 SC, Autostin 50 WDG, Filia 525 SE and DithaneM-45 were evaluated against mycelial growth of *Pyricularia oryzaein vitro* in the Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207. The highest inhibition of mycelial growth was observed when Potato Sucrose Agar (PSA) was amended with Trooper 75 WP (Tricyclazole) followed by Filia 525 EC and Nativo 75 WP.

Key words: *Pyricularia oryzae*, Fungicides, Rice blast, survey.

INTRODUCTION

Rice (*Oryza sativa*) is the staple food of Bangladeshi people and it constituted about 90% of the total food grain production (Huda 2001). It covers about 84.5% of the total cultivable land in Bangladesh (BBS 2008). The average world yield of rice is 3.84 t/ha, but the average yield of rice in Bangladesh is only 3.02 t/ha (FAO 2017). Rice is known to be attacked by many pests and diseases which cause huge annual losses worldwide. Among fungal diseases, rice blast caused by *Pyricularia oryzae* is of significant economic importance. Outbreaks of rice blast are a serious and recurrent problem in all rice growing regions of the world. It is estimated that 60 million people could feed each year by the produce of rice which is destroyed by rice blast (Zeigler *et al*. 1994).

Blast disease has been known since early 1600's and is cited in Chinese and Japanese literature as 'rice fever'. The disease was first recorded in China as seedling blight and the name blast was coined by Metcalf in 1907 (Manibhushan Rao 1994). Rice blast pathogen is widely distributed and highly destructive under favorable conditions. Temperature, moisture, sunshine, humidity and wind speed play a major role in the infection and development of the disease. Blast disease is caused by the fungus *Pyricularia oryzae* Cavara teleomorph: *Magnaporthe oryzae* B. Couch] (Couch and Kohn, 2002). Blast is the most destructive fungal disease affecting global rice production. In temperate flooded and tropical upland rice ecosystems rice blast cause significant yield loses (Shahjahan 1994). The fungus is known to occur in 85 countries worldwide. The International Rice Research Institute, Philippines, estimates that in order to feed the growing global population, rice production must be increased by another one-third by the year 2020.

Resistant cultivars and chemical fungicides are important in blast disease control. However, the durability of genetic resistance in improved rice cultivars is often short-lived in the field because the pathogen rapidly evolves to overcome resistance. The key to chemical control of rice blast disease is to overcome fungicide resistant pathogens.

MATERIALS AND METHODS

Experimental site

Survey was conducted in different districts of Bangladesh namely Mymensingh, Kishoreganj, Barishal, Naogaon and Cumilla (Fig. 1). Isolation of *Pyricularia oryzae*, determination of cultural characteristics and *in-vitro* study on efficacy of fungicides were tested in the Plant Pathology

laboratory of the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka -1207.

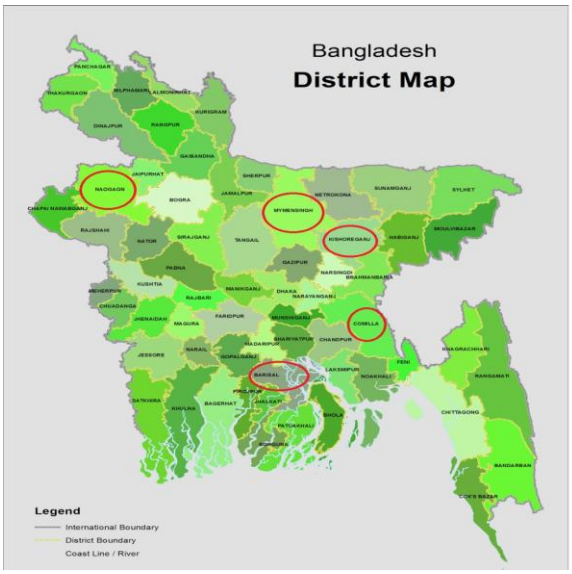

Fig. 1. Rice blast survey and sampling area in five districts of Bangladesh

Survey on incidence and severity of rice blast and collection of diseased sample

The survey on rice blast disease was conducted in farmers' fields of Bangladesh during Boro (November to May; irrigated ecosystem) season. Disease incidence and severity was recorded during survey of farmers' field. Disease incidence was recorded on the basis of the percent diseased panicle present in the field. Disease severity was recorded as 0 to 9 scale developed by International Rice Research Institute (IRRI 2014).

Culture medium used and preparation

Water agar (WA), Oat meal agar (OMA) and Potato Sucrose Agar (PSA) media were used for isolation, purification of the causal organism from the infected rice blast samples.

Water agar (WA) medium preparation

Seventeen gram of agar was put in a 2-liter capacity flask. 1000 ml distilledwaterwas mixed with agar in the flask. Then the flask was plugged with cotton and autoclaved for 15 min at 15 psi. (Hayashi*et al*, 2009).

Oat meal agar (OMA) preparation

Thirty gram of oat meal and 17 g agar was put in a 2-liter capacity flask and 1000 ml distilled water was mixed with agar in the flask. Then the flask was plugged with cotton and autoclaved for 15 min at 15 psi.

Potato sucrose agar (PSA) medium preparation

Two hundred gram of potato was boiled in 500 ml distilled water for one hour. After boiling, potato juice was filtrated through cheese cloth or nylon mesh and 20 g sucrose and 20 g agar was poured into the potato juice and the volume was adjusted to 1000 ml by adding distilled water. Finally, the flask was plugged with cotton and autoclaved for 15 min at 15 psi.

Isolation, purification, identification and maintenance of *Pyricularia oryzae*

Collected infected leaf and panicle samples were cut in 3-5 cm sections. These sections were surface sterilized by dipping in 10% Clorox for one minute and were washed by sterilized water for several times and then the cut sections were placed on moist filter paper (Whatman: 9.0 cm) in a sterile petri plates. Plates were incubated for 24 hours at room temperature (25^0C) and the infected parts were examined under stereo microscope (Motic SMZ-168). Conidial masses were picked by using very fine tip needle and spread on 3% water agar plate. The plates were observed under stereo microscope and single conidia picked by using needle and transferred it to another agar plate. That plate was incubated at 25^0C temperature for 2 or 3 days and finally the mycelium was transferred to potato sucrose medium plates (Hayashi *et al*. 2009). The marginal mycelial growth that developed subsequently was picked-up aseptically for sub-culturing. The sub culturing was done at an interval of 15 days and preserved at low temperature (5±1ºC) in refrigerator. When pure growth of the fungus was achieved, 5 mm culture discs of the fungal mycelium were cut with the help of sterilized cork borer and transferred aseptically in oat meal agar slants and allowed to grow. The pure culture slants were sealed with paraffin wax and stored in a refrigerator for further use. The pathogen isolated from the diseased specimen and established in pure form on OMA was identified on the basis of colony, morphological characters and pyriform shaped conidia.

Pathogenicity study

A pot culture technique was used to prove the pathogenicity of the test organism. The blast susceptible BRRI dhan28 seeds were sown in sterilized earthen pots containing sterilized soil + Farm Yard Manure (FYM) (1:1). The seedlings with vigorous growth were selected for artificial inoculation. Then the pathogen was inoculated by spraying over the seedlings @ 10^5 conidia/ml suspension. After spraying the seedlings were covered with polythine paper. The observations on the development of symptoms were recorded daily for a period of 15 days from the day of inoculation.

Mycelial growth and morphological characterization

Mycelial growth of *Pyricularia oryzae* on PSA was recorded at seven days after inoculation (DAI), 14 DAI and 21 DAI (Fig. 2). Data on spore morphology viz. shape, size and color of conidia were also determined on oat meal agar (Fig. 3 and Fig. 4). Sporulation ability of the *Po*isolates was determined following a rating system described by Meena (2005).

In vitro* efficacy of different fungicides against *Pyricularia oryzae

Poisoned Food Technique was applied in present assay for determination of efficacy of fungicides following Nene and Thapliyal (1993). Each fungicide with a control was tested against the *Pyricularia oryzae*. Potato sucrose agar was used as basal medium and distributed in 100 ml aliquots in each 250 ml Erlenmeyer flasks, which were sterilized at 15 lb psi pressure for 15 minutes. The quantity of fungicide per treatment was calculated for 100ml medium separately. The requisite quantity of test fungicide was added to each flask at 45°C. The fungicides were thoroughly mixed before solidification and poured immediately into sterilized Petri plates. The mycelial disc of 5 mm diameter of 15 days old culture was cut with the help of sterile cork borer. Each disc was transferred aseptically to the center of each Petri plate, already poured with poisoned medium. The PSA plates without fungicide (with water only) were also inoculated and maintained as control. The plates were incubated at 27 ± 1°C for seven days. Four replications per treatment were maintained. The observations on colony growth were recorded until Petri plate in control treatment was fully covered with mycelial growth. The details of the tested fungicides were presented in Table 1.

RESULTS AND DISCUSSION

The blast disease incidence and severity recorded during the survey is shown in Table 2. From the survey, the highest incidence of blast disease was recorded from Muktagachha (60%) with a severity of 5. The highest severity of blast disease was observed in Hossainpur, Kishoreganj (7) but percent incident was observed only 20%. The highest mycelial growth (79.50 mm) was observed in case of the isolates *Po*4 and the lowest (68.33 mm) in case of the isolates *Po*1 (Table 3). The mycelial growth of isolates *Po*3 and *Po*4 are statistically similar. The isolate *Po*3 showed the highest sporulation (4) and *Po*1 and *Po*4 showed the lowest (2). Eight fungicides belonging to different groups were tested *in-vitro* for their efficacy against *Pyricularia oryzae*, by employing poisoned food technique in PSA as basal medium.

Table 1. Details of fungicides tested in this study

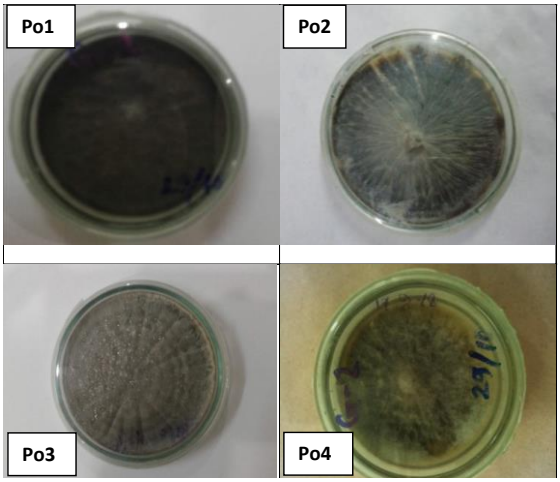

Fig. 2. Pure culture of four isolates of *Pyricularia oryzae* on PSA

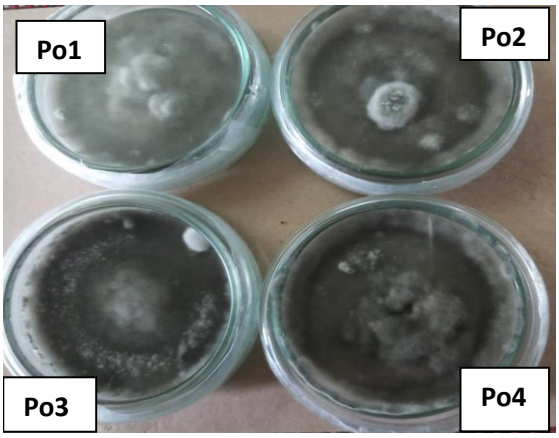

Fig. 3. Mycelial growth of four isolates of *Pyricularia oryzae* on OMA

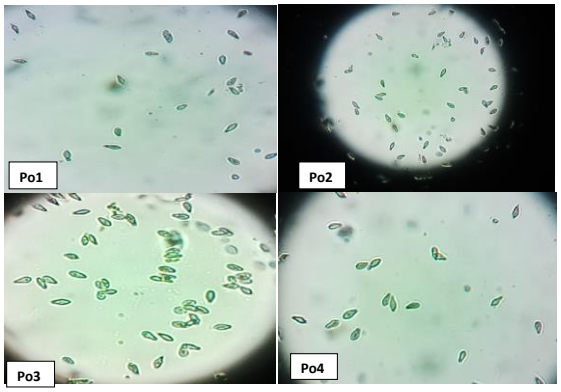

Fig. 4. Conidia of four isolates of *Pyricularia oryzae* on OMA observed under compound microscope

Trade name of Fungicides	Dose (ppm)	Active ingredient	Company name
Amistar Top 250 SC	100	Azoxystrobin + Difenoconazole	Syngenta Bangladesh
Autostin 50 WDG	100	Carbendazim	Auto Crop Care Ltd.
Azonil 56 SC	100	Azoxystrobin + Chlorothalonil	Haychem Bangladesh Ltd.
Dithane M-45	100	Mancozeb	Bayer Crop Science Ltd.
Filia 525 SE	100	Tricyclazole + Propiconazole	Syngenta Bangladesh
Nativo 75 WP	100	Trifloxystrobin + Tebuconazole	Bayer Crop Science Ltd.
Seltima 100 CS	100	Pyraclostrobin	BASF
Trooper 75 WP	100	Tricyclazole	Auto Crop Care Ltd.
Control (Only water)	-	-	-

Table 2. Survey on incidence and severity of rice blast disease in different districts of Bangladesh in Boro season 2017-2018

Name of districts		Name of Upazillas	Available Varieties	No. field visited	Blast disease	
					Incidence (%)	Severity Score
Mymensingh		Gafargaon	BRRI dhan28,BRRI dhan81	7	30	5
		Bhaluka	BRRI dhan28, BRRI dhan63	10	20	3
		Muktagacha	BRRI dhan29,BRRI dhan81	15	60	5
Kishoreganj		Hossainpur	BRRI dhan28	8	20	7
		Mithamain	BRRI dhan29	10	20	5
		Pakundia	BRRI dhan28, BRRI dhan63	6	30	5
Barishal		Mehendigaj	BRRI dhan74, BRRI dhan89	5	10	3
		Babuganj	BRRI dhan74	10	5	3
		Bakerganj	BRRI dhan89	12	5	1
Naogaon		Manda	Ziradhan, BRRI dhan63	8	20	5
Cumilla		Chandina	BRRI dhan28, BRRIdhan29	10	50	5
		Burichang	BRRI dhan28, BRRI dhan29	9	30	3
Total	5	12	7	110		

Table 3. Mycelial growth and sporulation of four isolates of *Pyricularia oryzae* on oat meal agar

Isolates of *P. oryzae*	Mycelial growth (mm)	Sporulation Index	
		Index	Sporulation Type
Po 1	68.33c	2	Fair
Po 2	74.83b	3	Good
*Po*3	77.67a	4	Excellent
Po 4	79.50a	2	Fair
CV (%)	1.88		

In-vitro mycelia growth at different treatment was found significantly different (Table 4). Maximum growth inhibition of *Pyricularia oryzae* was achieved with Trooper 75 WP (80.29%), Filia525 SE (76.19%) and Nativo 75 WP (71.15%), which was significantly different and superior to rest of the treatments.

Joshi and Mandokhot (2002) conducted a field trial to determine the efficacy of Tricyclazole 75 WP and found the same results at different concentrations for controlling blast of rice. Vijay (2002) also tested the efficacy of Tricyclazole (0.1%) against rice blast and found the reduction in disease incidence of 17.6%.

In-vitro mycelial growth of *Pyricularia oryzae* on different combinations at 14 DAI was found significantly different. The maximum inhibition of *Pyricularia oryzae* was achieved with Trooper (76.72%), Filia (73.48%) and Nativo (67.89%), which

was significantly different and superior to rest of the treatments. Trooper (76.72%) was found to be the best effective fungicide. These results are in agreement with those reported by Hossain and Kulkarni (2001) and Bhojyanaik and Jamadar (2014).

In-vitro mycelial growth of *Pyricularia oryzae* on different combinations at 21 DAI was found significantly different (Table 4). The maximum inhibition of *Pyricularia oryzae* was achieved with Trooper 75 WP (71.46%), Filia525 SE (71.26%) and Nativo 75 WG (64.57%), which was significantly different and superior to rest of the treatments. Trooper 75 WP (71.46%) was found to be the best effective fungicide. The results are in concurrence with those of Joshi *et al.* (2014) and Bhojyanaik and Jamadar (2014).

CONCLUSION

From the above result it can be concluded that Trooper 75 WP was found to be the best effective fungicide in controlling mycelial growth of *Pyriculariaoryzae in-vitro.*

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Table 4. Effect of fungicides on mycelial growth and percent inhibition *in-vitro* at 7, 14 and 21 days after inoculation (DAI)

Treatments	Mycelial growth and inhibition					
	7 DAI		14 DAI		21 DAI	
	Mycelial growth (mm)	Growth inhibition over control (%)	Mycelial growth (mm)	Growth inhibition over control (%)	Mycelial growth (mm)	Growth inhibition over control (%)
Trooper 75WP	10.25 f	80.29	13.5 h	76.72	18.12f	71.46
Filia 525SE	12.38 ef	76.19	15.38 gh	73.48	18.25 ef	71.26
Nativo 75 wp	15.00 e	71.15	18.62 fg	67.89	22.50 de	64.57
Amister Top 250SC	24.75 c	52.40	28.38 d	51.07	32.75 c	48.43
Azonil 56SC	18.75 d	63.94	21.98 ef	62.10	26.0d	59.06
Dithane M-45	37.75 b	37.75	41.12 b	29.10	45.12 b	28.94
Autostin 50WDG	25.62 c	50.73	35.5 c	38.79	41.12 b	35.24
Seltima 100CS	18.38 d	64.65	22.75 e	60.78	26.62 d	58.08
Control	52.00 a	-	058 a		63.50 a	-
±SE	1.63	-	1.86		2.11	-
CV(%)	9.64	-	9.29		9.14	-

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