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Determining an effective and economic fungicide spray schedule for reducing blast of wheat

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ABSTRACT

Wheat blast, caused by the fungus *Magnaporthe oryzae pathotype* Triticum (MoT), constitutes one of the major obstacles to the expansion of wheat production in Bangladesh. In the absence of resistant variety, fungicide control is the first-hand effort. Determining an effective and economic fungicide spray schedule in controlling blast disease of wheat was aimed. Ten fungicides were tested during two consecutive cropping seasons of 2018-2019 to 2019-2020. The wheat plants of blast susceptible cultivar BARI Gom 26 were inoculated with spores (10^7 spores ml^{-1}) of MoT at pre-heading stage of wheat (52 days age). Fungicides were applied both before inoculation and after the appearance of blast symptoms in cocktail for three times starting from booting of wheat at 7 days interval. Plants received the combination of Filia (Tricyclazole 40% + Propiconazole 12.5%) and Seltima (Pyraclostrobin 10%) had significantly lower blast incidence and severity (1.23% and 3.33%) against untreated plants. Cocktail of Nativo and Trooper (Tricyclazole 75 wp) proved 2nd best curative measure. Application of Nativo (Tebuconazole 50% + Trifloxystrobin 25%) alone ranked third in its efficacy. The fungicide spray schedule covered booting, pre-heading and heading stages of wheat. The results indicate a mixture of Tebuconazole + Tricyclazole + Pyraclostrobin is more effective (97% blast reduction) and economic (BCR 1.45) than a single compound application in reducing incidence and severity of wheat blast.

Keywords: Wheat blast, Fungicide spray schedule.

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Introduction

Wheat is the second most important staple food crop in Bangladesh after rice (Karim *et al.*, 2010). Within a period of 30 years of time, it has been firmly established as a secure crop in Bangladesh, mainly due to stable market price and involvement of huge farmers. With the emergence of new biotic cause, an added stress to climate change and the rapidly declining per capita arable land, meeting the demand to supply of wheat is increasingly challenging and threatening to food production and food security in developing country like Bangladesh (World Bank, 2016). In 2016, the devastating wheat-blast disease caused by the fungus *Magnaporthe oryzae* pathotype Triticum (MoT) was reported in Bangladesh (Callaway, 2016).

Blast attacks the rachis, leading to bleached spikelets above the point of infection and bright black spots on the rachis (Duveiller *et al.*, 2016). Grains from blast-infected heads are usually small, shriveled and deformed. The highest yield losses happen when head infections start during anthesis or early grain development stages. At present, most of the commercially grown wheat cultivars in South Asia are susceptible to wheat blast, BARI Gom 33 released as blast resistant variety is in farmer's field (Hossain *et al*, 2019). The level of yield losses and speed of epidemics caused by MoT along with the lack of resistance may require innovative approaches to manage this disease. Chemical fungicides spray remains the main approach for controlling wheat blast until a dependable resistant cultivar is developed.

Application of single chemical compound such as tebuconazole or tricyclazole or trifloxystrobin proved not effective in reducing blast disease severity (Urashima *et al.*, 2009). Mixture of two of these compounds when applied produced better results (Valent *et al.*, 2016; Rios *et al.*, 2016), may be combination of all the three compatible compounds might yield significantly better results. Other important side is that these fungicides acted well when applied before the appearance of blast disease as preventive measure, not providing curative action once the spike started bleaching. Therefore, the present study was designed for finding out fungicide(s) and an effective spray schedule as both preventive and curative measures against incidence and severity of wheat blast.

Materials and Methods

Experimental site

The experiments were conducted during the cropping seasons of 2018-19 and 2019-20 in the laboratory and net house of Plant Pathology Department, Bangladesh Agricultural University, and Plant Pathology Division, Bangladesh Institute of Nuclear Agriculture. Blast susceptible wheat variety BARI Gom 26 was used in the research work.

Fungicides used in the experiment

Ten fungicides collected from local market and marketing industries were used (Table 1) in the experiment.

Table 1. Fungicides used in the experiments.

Treatments	Name of Fungicides	Groups	Concentration used in bioassay (ppm)	Doses applied* in the net house
F_0	Control	-	00.00	00.00
F_1	Nativo	Tebuconazole 50% + Trifloxystrobin 25% w/w WG	600	6 g 10L^{-1}
F_2	Filia	Tricyclazole 40% + Propiconazole 12.5%	2000	20 ml 10L^{-1}
F_3	Seltima	Pyraclostrobin 10%	2000	20 ml 10L^{-1}
F_4	Kaicin	Kasugamycin 3% + Tricyclazole 77%	500	5 g 10L^{-1}
F_5	Edifen	Edifenphos 50%	1700	17 ml 10L^{-1}
F_6	Diaben (Amistar Top)	Azoxystrobin 20% + Difenoconazole 12.5%	1000	10 ml 10L^{-1}
F_7	Trooper	Tricyclazole 75 WP	800	8 g 10L^{-1}
F_8	SunFighter	Hexaconazole 3% + Tricyclazole 22%	2000	20 ml 10L^{-1}
F_9	Score	Difenoconazole 25%	2000	20 ml 10L^{-1}
F_{10}	Provax 200 WP	Carboxin 17.5% + Thiram 17.5%	0.03	3 g kg^{-1} seed

**Doses as recommended for commercial application.*

Bioassay of fungicides

The linear growth (cm) and % growth inhibition of mycelium of *Magnaporthe oryzae* Triticum (MoT) were observed *in vitro* by poisoned food technique (Nene and Thapliyal, 1979) (Table 2).

In this Technique, fungicide solution was prepared by dissolving requisite quantity of chemical in sterilized water. From PDA plate, three 5.0 mm discs of the medium was scooped off three places maintaining an equal distance from the centre by a sterilized disc cutter. One milliliter of fungicides solution was put into each hole and the plates were stored overnight in refrigerator for diffusion of the input in the medium around the hole. The next day, one 5 mm culture block of MoT (20 days old) was cut and placed at the centre of the treated PDA plate. For control treatment, only sterile water was used instead of fungicides. The plates were then placed at 30±1°C for 20 days.

Measurement of growth of MoT and % growth inhibition

The linear growth (cm) of mycelium of MoT was recorded at 2 days interval until the control plates were filled in. Efficacy of the treatments in inhibiting radial mycelial growth of MoT *in vitro* was determined by the following formula:

$$\text{Mycelia growth inhibition } (\%) = \frac{\text{Mycelia growth (dia)in the treated plate(mm)}}{\text{Mycelia growth in the control plate (mm)}} \text{ x } 100$$

Preparation of experimental pot and sowing of seed

Each of the plastic pots were filled up with 16 kg silt-loamy soil. Thirty (30) seeds were sown on 30 November 2018 and 2019 in each of the prepared pots. Thinning was done at 20 days after sowing (DAS) to maintain 15 plants per pot. The fertilizers were applied in each pot as per the Fertilizer Recommendation Guide (BARC, 2018). Weeding and watering was uniformly done when required.

***Culture of M. oryzae Pathotype* Triticum**

Pure culture of MoT was collected from IPM Lab, Bangladesh Agricultural University. The culture was multiplied in Oatmeal agar media. The plates were incubated at 30 ± 1°c with continuous NUV light (650 lux) for 15-20 days for sporulation (Fig. 1). Density of the spores was calculated by harvesting the conidia/mycelia by flooding the Petri dish with 5 ml of sterile distilled water and dislodging the conidia with a bent glass rod. Spore density was 1 x 10^7 per ml as it was calculated in Hemocytometer count. The germination ability of the spores was checked through continued microscopic observation of the slide prepared out of spore suspension.

Inoculation of the test pathogen (MoT)

Wheat plants of blast susceptible variety BARI Gom 26 grown in pot soil were inoculated with *Magnaporthe oryzae* Triticum spores @ 10^7 CFU. Wheat plants at the age of 52 days i.e. pre-heading stage were inoculated. After inoculation, the plants were kept covered under polythene shed for 48 h to maintain high humidity (>80% RH) and temperature 30±1°C. Observations were made for the expression of blast disease symptoms. Isolation of the causal organism was made from infection court for the confirmation of successful infection by *Magnaporthe oryzae* Triticum (Fig. 1).

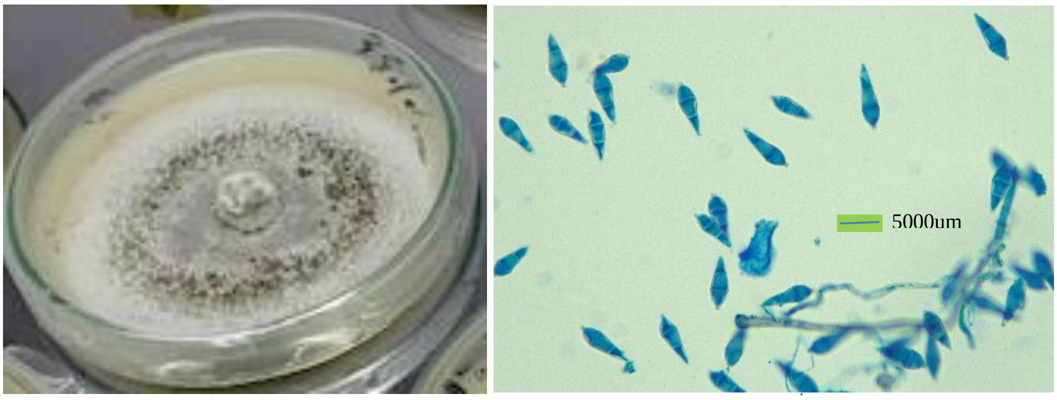

Fig. 1. Culture (20 days old) and typical 2-septate 3-celled pyriform spores of *Magnaporthe oryzae* Triticum (Microscopic view 40x).

Spraying fungicide-suspension

As preventive measure, first spray of fungicides was given at booting stage (47 days age) of wheat plants i.e. before the appearance of any blast symptoms. Two sprays during 2018-19 and three sprays during 2019-20 cropping seasons were applied at 10 days interval. The third spray during 2019-20 served as curative measure as was given on-sight blast symptoms i.e. 14 days after inoculation with MoT, at 66 days age of the wheat plant.

Treatments and design of experiment

In the cropping season of 2018-19, eight fungicides each at single doses were used. There were nine treatments including control. During the cropping season of 2019-20, seven fungicides selected out of previous year's trial including two new were applied either single or mixture of two at single doses of each. There were eight treatments including control. The treatments were set following completely randomized design (CRD) with three replications.

Data collection

The incidence of wheat blast and its severity were scored for four times during 2018-19 and five times during 2019-20 experiments at two days interval starting at 68 days age of the plants i.e. 2 days after symptoms expression.

Disease Incidence: Number of spikes infected per replication expressed in percentage (Rajput and Bartaria, 1995). % blast incidence = Pi/Pt x 100 where, Pi = Number of spikes infected, and Pt = Total number of spikes counted.

Disease Severity: Percent area of spike infected/bleached was estimated. Spikes per pot were counted and arithmetic means for single plants were calculated. Disease severity was scored following the figures shown below given by Maciel *et al.* (2013): 0 = No lesion, 1 = 25% or less, 2= 26-50%, 3= 51-75% and 4= 76-100%.

Statistical analysis of data

The data were statistically analyzed using Minitab 18 computer package program and means were compared by DMRT (Duncan's Multiple Range Test).

Results and Discussion

Bioassay of fungicides against Magnaporthe oryzae Triticum

Out of eight fungicides, Nativo, Sunfighter, Filia and Trooper completely inhibited the mycelial growth of *M. oryzae* Triticum (MoT). Other seven arrested the growth of MoT at different levels (Fig. 2).

Nativo Filia SunFighter Trooper Control

Fig. 2. Growth of *Magnaporthe oryzae* Triticum on PDA poisoned with fungicides at 20 days after inoculation.

Spraying fungicides at booting stage of wheat

Symptoms appeared approximately 14 days after inoculation in untreated (control) plants. The treated plants also showed blast infection almost at the same time. Typical blast symptoms of spike bleaching advancing from top to downward were observed (Fig. 3).

Fig. 3. Typical spike bleaching of wheat plants of variety BARI Gom26 inoculated with *Magnaporthe oryzae* Triticum in the BINA net house.

Both blast incidence and severity were monitored for 12 days from the 2nd day of the on sight of the blast disease. In the cropping season 2018-19, none of the fungicides displayed any good results. Nativo, Sun Fighter and Filia treated plants had 33, 30 and 27% less incidence of blast, respectively. During 2019-2020, wheat plants receiving no fungicide sprays had a continued increase in spike bleaching showing 100% blast incidence on the 80 days age. Blast incidence either remained static or increased slowly on the wheat plants sprayed with fungicides. Seed treatment did not have any effect on the incidence of wheat blast (Table 2).

Wheat plants treated with single, or mixture of fungicides had significantly different levels of blast incidence. F_5 (Filia@ 2.0 ml L^{-1} + Seltima @ 2.0 ml L^{-1}) treated plants didn't show blast symptoms up to 70 days age, only 1.23% plants had bleached spike finally. Wheat plants treated with other six fungicides showed blast symptoms from 68 days age. F4 and F1 treated plants had significantly lower blast incidence of 6.71 and 8.12%, respectively. As per the performance of disease incidence, spraying cocktail of F5 (Filia @ 2.0 ml L^{-1} + Seltima @ 2.0 ml L^{-1}) was found the best where 98.77% reduction of disease was observed over control at 80 DAS. The blast incidence recorded for the spraying of F4 (Nativo + Trooper) and F1 (Nativo) were statistically similar to that of F5. So, F4 treatment i.e., mixture of Nativo and Trooper proved as the second-best treatment (Table 2).

Table 2. Effect of three sprays of fungicide as preventive measure starting from booting stage in reducing incidence of wheat blast in the net house of BINA during 2019-2020 cropping season.

Treatments	Disease Incidence (%)				
	68 DAS	71 DAS	74 DAS	77 DAS	80 DAS
F_0	30.00 a	38.30 a	54.03 a	78.89 a	100.00 a
F_1	3.36 b	4.75 b	4.75 b	4.75 c	8.12 cd
F_2	4.303 b	5.493 b	5.510 b	6.793 c	9.930 cd
F_3	4.517 b	5.800 b	7.650 b	9.500 c	12.750 c
F_4	3.00 b	3.00 b	4.33 b	4.33 c	6.713 cd
F_5	0.00 b	0.00 b	1.23 b	1.23 c	1.23 d
F_6	5.89 b	5.89 b	8.93 b	8.93 c	11.96.cd
F_7	26.41 a	33.01 a	46.22 a	60.67 b	85.55 b
CV (%)	33.91	43.46	33.06	17.92	10.21
LSD(0.05)	7.48	13.62	12.84	7.67	6.56

F_0 = 0.0 (Tape water), F_1 = Nativo @ 0.6 g L^{-1}, F_2 = Filia @ 2.0 ml L^{-1}, F_3= SunFighter @ 2.0 ml L^{-1}
F_4 = Nativo @ 0.6 g L^{-1} + Trooper @ 0.8 g L^{-1}, F_5 = Filia @ 2.0 ml L^{-1} + Seltima @ 2.0 ml L^{-1}
F_6= SunFighter @ 2.0 ml L^{-1} + Score 250EC @ 1.0 ml L^{-1}, F_7 = Provax @ 3g kg^{-1} seed.
Figures in a column with different letters are significantly different. DAS: Days After Sowing.

Severity of blast disease was significantly reduced for fungicide sprays. F5 treatment i.e., cocktail of Filia and Seltima spray reduced blast severity by 97% over untreated. Treatments F4 and F1 displayed statistically similar effect, reduced blast severity by 87 and 82%, respectively (Table 3). Seed treatment did not have any effect on spike bleaching of wheat.

Yield and BCR

Plants treated with Filia + Seltima (F5) and Nativo + Trooper (F4) had >4 ton/ha of wheat yield which was significantly higher than all other treatments and the control. A BCR of 1.45 was obtained from the F5 (Filia + Seltima) treatment. Second highest BCR was recorded for F4 (Nativo + Trooper) treatment (Table 3). Bleaching of spikes by wheat blast infection reduced wheat yield by 93.9% (Table 3).

Table 3. Effect of three sprays of fungicide as preventive measure starting from booting stage in reducing severity of wheat blast in the net house of BINA during 2019-2020 cropping season.

Treatments	Disease Severity (%)					Yield	Benefit Cost
	68 DAS	71 DAS	74 DAS	77 DAS	80 DAS	(ton ha^{-1})	Ratio(BCR)
F_0	44.67 a	58.33 a	71.67 a	79.33 a	98.00 a	0.2767 e	0.092
F_1	10.67 b	11.33 b	12.00 b	12.67 b	17.33 b	3.6000 b	1.160
F_2	8.33 b	10.00 b	15.00 b	18.67 b	27.67 b	3.1340 bc	1.009
F_3	6.00 b	8.00 b	16.67 b	23.00 b	32.70 b	2.9670 c	0.950
F_4	6.00 b	7.33 b	7.33 b	7.33 b	13.00 b	4.1930 a	1.350
F_5	0.00 b	0.00 b	1.67 b	2.67 b	3.33 b	4.5210 a	1.450
F_6	9.33 b	12.33 b	15.33 b	20.00 b	29.33 b	3.0670 bc	0.990
F_7	42.00 a	55.67 a	60.33 a	68.00 a	86.33 a	0.9660 d	0.318
CV (%)	48.73	31.64	34.93	27.96	23.40	9.63	-
LSD(0.05)	16.87	13.51	16.53	16.29	19.25	0.547	-

F_0 = 0.0 (Tape water), F_1 = Nativo @ 0.6 g L^{-1}, F_2 = Filia @ 2.0 ml L^{-1}, F_3= SunFighter @ 2.0 ml L^{-1}
F_4 = Nativo @ 0.6 g L^{-1} + Trooper @ 0.8 g L^{-1}, F_5 = Filia @ 2.0 ml L^{-1} + Seltima @ 2.0 ml L^{-1}
F_6= SunFighter @ 2.0 ml L^{-1} + Score 250EC @ 1.0 ml L^{-1}, F_7 = Provax @ 3g kg^{-1} seed.
Figures in a column with different letters are significantly different, DAS: Days After Sowing.

Both the blast incidence and severity increased with time in both the untreated plants (F_0) and plants sprayed with fungicides. Similar trend was observed in plants raised from Provax treated seeds (F_7). In case of plants sprayed with cocktail of Filia and Seltima (F_5), Nativo and Trooper (F_4) and Nativo alone (F_1), incidence and severity of spike blast had a reduced rate of increase (Figure 4). On the other hand, seed treatment with fungicide could not prevent blast infection or stop its motion. At 80 days of the crop, blast incidence and severity reached nearly to 100% in the untreated plants while at this time, the plants sprayed with the cocktail of Filia and Seltima had blast incidence and severity of 1.23 and 3.33%, respectively (Fig. 4). F5 treated plants did not show blast infection up to 71 days age of the crop.

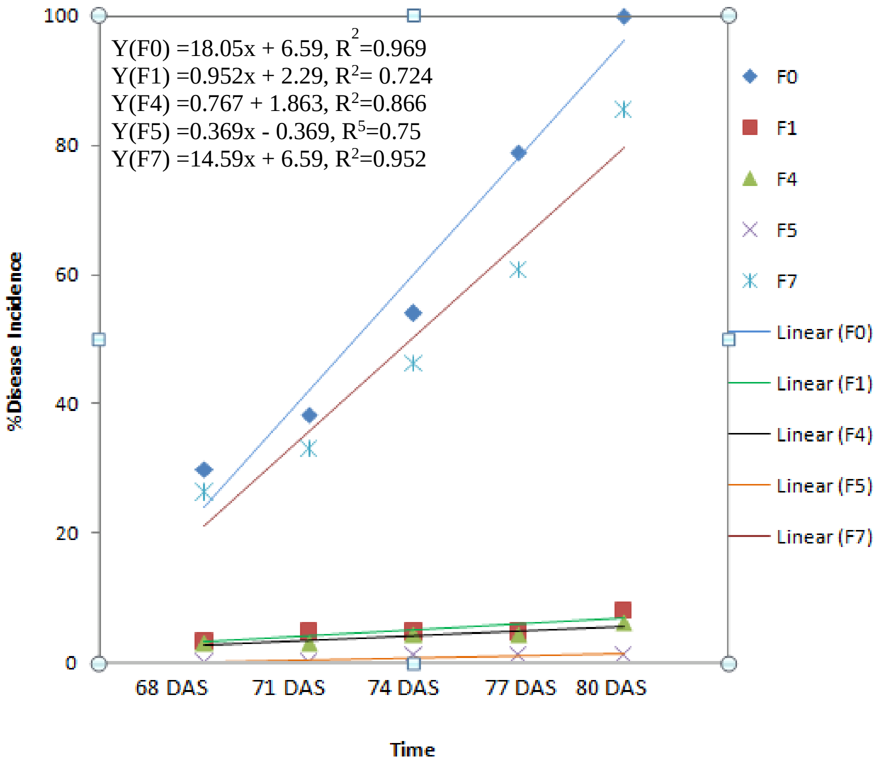

Fig. 4. Reduction of incidence of wheat blast as intervened by spray of cocktail of fungicides. DAS: Days After Sowing, F_0: untreated, F_1: Nativo, F_4: Nativo + Trooper, F_5: Filia + Seltima, F_7: Seed treatment with Provax 3 g kg^{-1} of seed.

Wheat blast appeared with fears in Bangladesh in 2016, had been fearsome to Brazil and Bolivia since 1985. The production loss is 100% if spikes were infected at heading stage (Hossain *et al.*, 2019). Management of wheat blast is, therefore, a must. Because wheat shares a vital role in attaining food security of Bangladesh and countries like Brazil, Bolivia, Nepal, Pakistan and India (Duveiller *et al.*, 2016). In the absence of durable blast resistant variety, other means of management practices such as fungicides, nutrient supplementation are in the front to adopt. Blast pathogen has also evolved to acquire resistance to fungicides extensively used to manage the disease (Oliveira *et al.*, 2015). However, present findings concede the opinion of Bockus *et al.* (2014) that effective fungicides spray can reduce MoT sporulation by 52.2 to 100%.

In the present investigation, perfect control of wheat blast could not be achieved. It might be because of the insufficient protection given by only one spray of the fungicides at heading stage. This finding partially agrees with Valent *et al.* (2016) who found combined application of triazoles effective in controlling wheat blast in heading stage in moderately resistant variety. Continued spray could bring better result, but it would cause increase in production cost.

Solo spray of Trooper, Filia, Seltima, Nativo and SunFighter could not stop blast infection motion. However, combined use of Filia and Seltima, and Nativo and Trooper brought the blast incidence and severity to a satisfactory level yielding a good return of wheat yield. The results indicated the combined spray of Tricyclazole, propiconazole and pyraclostrobin was the best curative measure. The results are supported by Rocha *et al.* (2014) who reported considerable reduction of wheat blast infection through use of pyraclostrobin and trifloxystrobin.

Three sprays of combined application of Filia and Seltima covered the three blast vulnerable growth stages of booting, pre-heading and heading served as both preventive and curative measures. Spore inoculation was done at pre-heading stage, those spores failed to germinate and penetrate into host tissues in the presence of the fungicide. Second spray at pre-heading stage might have killed spores from any external source. Third spray at heading stage kept the emerging spikes free of blast infection. This approach reduced blast infection by 97% and gave a very good yield, >4.5 ton ha^{-1} which is higher than national average of 3.6 ton ha^{-1} in Bangladesh (BBS, 2019).

Our research indicates both Filia + Seltima mixture and Nativo + Trooper mixture are equally effective in wheat blast disease control. The third choice is solo application of Nativo. These results will help farmers to alternatively using any one of the three groups of fungicides just avoiding repetition, a right step to combat the mutation effect of *Magnaporthe oryzae* Triticum if any (Castroagudin *et al.*, 2015; Oliveira *et al.*, 2015).

Conclusion

Three sprays of cocktail of (Tebuconazole + Propiconazole + Pyraclostobin) beginning from booting of wheat reduced blast severity by 97% on susceptible wheat variety and produced >4.5 ton ha^{-1} yield with BCR 1.45.

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