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Screening of Different Fungicides in Controlling Wheat Blast in Bangladesh

J. Farthouse[1*], M.A. Kashem[1], M.K. Hasna[1], M.I. Khalil[1], M.M. Haque[1] and K.M.E. Nabi[1]

[1]Plant Pathology Division, Bangladesh Institute of Nuclear Agriculture, Mymensingh, Bangladesh
*Correspondence: jannat.topy@yahoo.com, Tel: +8801703808002

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Abstract: Wheat blast caused by *Magnaporthe oryzae* pathotype *Triticum* (MoT) is an aggressive fungal disease threatening wheat production in Bangladesh. In the absence of resistant varieties, fungicide control is a first-hand effort. This study aimed to evaluate the performance of different fungicides against wheat blast in pot conditions and to identify the best one for preventive spray. The efficacy of five fungicides i.e., Nativo 75WG (Tebuconazole 50% + Trifloxystrobin 25%), Trooper 75 WP (Tricyclazole), Two in One (Hexaconazole 3% + Tricyclazole 22%), Opponent 75 WG (Tebuconazole 50% + Trifloxystrobin 25%) and Azonil 56 SC (Azoxystrobin 6% + Chlorothalonil 50%) against wheat blast was evaluated. The blast susceptible variety BARI Gom 26 was planted in pot following CRD with three replications. Wheat plants (52 days old) were inoculated with spore suspension (10^7 CFU) from 7 days old MoT cultures grown on oat meal agar at 25°C. Fungicides were sprayed at the pre-heading stage (52 DAS) after 48 hours of inoculation. Data on disease incidence and severity were scored for seven times after first appearance of disease symptoms. The results indicate Nativo exhibited the highest level of fungicidal activity against blast with a 40.10% disease incidence and 91.81% severity, followed by Trooper with a 47.36% incidence and 97.45% severity. The diseases incidence for the rest three fungicides ranged from 53.90% to 85.10%. Nativo and Trooper showed 52.88 % and 44.35 % blast reductions respectively. It can be concluded that Nativo and Trooper fungicides can be used to control wheat blast by spraying twice at the 10 days interval at pre-heading stage.

Keywords: Wheat blast; Fungicides; Incidence; Severity.

INTRODUCTION

Wheat is the second most-produced cereal after maize in world (FAOSTAT 2020). In Bangladesh, it is the second most important staple food after rice (Karim et al. 2010). But in recent years wheat production has been threatened due to an emerging disease named blast. Wheat blast caused by a hemibiotrophic filamentous fungus *Magnaporthe oryzae Triticum* (MoT) pathotype is arguably the most yield-limiting wheat disease worldwide. It can acquire grain yield loss of up to 100% in different part of the world, including South America and Bangladesh depending on the disease development which is dependent on climatic condition prevailing throughout the cropping season, cultivar vulnerability, planting time, rainfall, relative humidity, etc. (CIMMYT 2016; Islam et al. 2016). In February 2016, Bangladesh was conveyed as the first Asian country to have an outbreak of annoying wheat blast disease (Callaway 2016; Islam et al. 2016). Seven western and southern districts of Bangladesh faced with significant yield damage due to WB (Malaker et al. 2016). The disease also observed in the subsequent years (2017 to 2020) with lower intensity but spread to several other (13 districts) wheat growing areas of the country (Roy et al, 2020). The pathogen affects all aerial parts of the wheat plant including spikes, leaves, leaf sheaths, stems, awns, grain, etc. but the most characteristics symptoms is observed in the spikes, which become bleached above dark lesions formed in the rachis, where the fungus sporulates abundantly.

The most effective, economic and environment friendly method to control this disease is the development of wheat blast resistant varieties. Although there is no proven commercial variety resistant to wheat blast, however, application of chemical fungicides can be considered as an interim measure for controlling the disease (Roy et al. 2018). Wheat growing farmers usually use chemical fungicides to manage the diseases, although their efficacy is variable. Foliar fungicide application yields good results to a point, but is ineffective under high disease pressure and only partially effective under moderate to low disease pressure (Kohli et al. 2011). In Bangladesh, several fungicides have been tried to control wheat blast but none can provide sufficient protection under highly conducive conditions. Therefore, the present study was designed to evaluate the fungicidal activity of fungicides for effective control of wheat blast under pot conditions favorable for blast progress.

MATERIALS AND METHODS

Experimental site

The experiments were carried out in the laboratory and net house of Plant Pathology Division, Bangladesh Institute of Nuclear Agriculture, Mymensingh, Bangladesh during the cropping seasons of 2018-19 and 2019-20 using blast susceptible wheat variety BARI Gom 26 (Figure. 1.).

Experimental design and treatment factors

The experiments were laid out in pot following completely randomized design with three replications and five treatments. Each of the plastic pot was filled up with16 kg silt-loam soil. Then thirty (30) seeds were sown on 30 November 2018 and 2019 in each of the prepare pots. Thereafter, thinning, weeding and watering were done whenever required. The fertilizers were applied in each pot according to Fertilizer Recommendation Guide (BARC, 2018). Five fungicides were collected from local market (Table 1).

Table 1. List of fungicides used in the experiment

Fungicides name	Chemical name/active ingredient	Concentration used in bioassay (ppm)	Recommended dose
Control	-	0.00	0.00
Nativo	Tebuconazole 50%+Trifloxystrobin 25% w/w WG (75WG)	0.04% = 400 ppm	0.4 g/L
Trooper	Tricyclazole 75 WP	0.075% = 750 ppm	0.75 g/L
Two in one	(Hexaconazole 3% + Tricyclazole 22%) 25 SC	0.1% = 1000 ppm	1 ml/L
Opponent	(Tebuconazole 50% + Trifloxystrobin 25%) 75 WG	0.04% =400 ppm	0.4 g/L
Azonil	(Azoxystrobin 6% + Chlorothalonil 50%) 56 SC	0.1%=1000 ppm	1 ml/L

Bioassay of fungicides

An *in-vitro* test was conducted to determine effectiveness of the fungicides on radial mycelial growth of *Magnaporthe oryzae* Triticum (MoT) following Complete Randomized Design (CRD) with four replications by poisoned food technique (Nene and Thapliyal, 1979). In this technique, fungicides solution was prepared by dissolving requisite quantity of chemical in sterilize water. Then the concentrated fungicide solution was incorporated and mixed well in conical flask containing Potato Dextrose Agar media at about 50ºC and poured 20 ml in each Petridish of 9 cm diameter. The freshly growing mycelium from the selected culture plate was cut 5 mm with cork borer and inoculated at the centre of the Petri dish under aseptic condition in an isolation chamber. Control was also maintained without any fungicides. Then the plates were incubated at 30±1ºC for 20 days.

Mycelial radial growth was taken in 24 hrs, 48 hrs, 3 days and 20 days after inoculation for all treatments and the inhibition percent of mycelium was calculated using the following formula given by Vincent (1927). Efficacy of the treatment in inhibiting radial mycelial growth of MoT *in-vitro* was determined by the following formula:

$$I\ (\%) = \frac{C - T}{C} \times 100$$

Where I is the percent inhibition, C is the Mycelia growth (dia) in the control plate (mm), and T is the Mycelia growth (dia) in the treated plate (mm)

Pathogen culture, inoculation and fungicide spray

The pathogen was isolated from infected spike of wheat collected from the field of Bangladesh Institute of Nuclear Agriculture. Then pure culture was made and the culture was multiplied in Oat meal agar media. The plates were incubated at 25°C with continuous NUV light (650 lux) for 15-20 days for sporulation. 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. The density of spore was 1 x10^7 per ml. Wheat plants were inoculated with spore suspension (10^7 CFU) at the age of 52 days i.e., pre-heading stage. After inoculation, the plants were kept covered under polythene shed for 48 h to maintain high humidity (>80% RH) and temperature 30±1°C. In pot, fungicides were sprayed at pre-heading stage (52 DAS) after 48h of inoculation with spores of *M. o. Triticum*. Two sprays were applied according to recommended dose during each cropping seasons (2018-19 and 2019-20) at 10 days interval. Observations were made for the expression of blast disease symptoms.

Data collection and analysis

Data on blast incidence and its severity were scored for seven times starting at 68 days age of the plants during both cropping season at two days interval. Blast incidence was calculated as the rate of symptomatic spike over total no. of spike (healthy and symptomatic) in percentage points. Severity was estimated according to the scale proposed by

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Trindade et al. (2006), which assigns notes according to the point at which the pathogen has penetrated the rachis, and consequently affects the area of the spike above the points of entry, where 0= symptoms absent, 1= 25% of the spike symptomatic, 2= 50% of the spike symptomatic, 3= 75% of the spike symptomatic and 4=100% of the spike symptomatic. Productivity per hector was estimated based on grain yield in treated and control plots. Data were analyzed statistically using Minitab 18 computer package program and means were compared by DMRT (Duncan's Multiple Range Test).

RESULTS AND DISCUSSIONS

Efficacy of the treatments in inhibiting radial mycelial growth of *Magnaporthe oryzae Triticum in-vitro* was determined.

Bioassay of fungicides against *Magnaporthe oryzae Triticum*

Figure 1 shows the inhibitory effect of five fungicides at 20 days after inoculation. The summary of mycelial growth data and inhibition of radial mycelial growth of MoT over control is shown in Table 2. MoT took 20 days for fully grown in control plates that are why data were taken till 20 days after culturing. Among the five fungicides tested in the present study, percent inhibition of mycelial growth in the PDA containing Nativo and Trooper was 100% from 24 hours to 20 days after culturing which was followed by Azonil (100 to 82 %), Opponent (100 to 80 %) and two in one (100 to 45 %). On the other hand, control was appeared badly in controlling mycelial growth. The maximum mycelial growth of the fungus i.e., colony diameter was recorded in control (90.0 mm) plate, where only sterile water was used instead of fungicides. The lowest colony diameter found in Nativo (0.00 mm) and Trooper (0.00 mm) treated plates which was followed by Azonil (16.20 mm) and Opponent (18.00 mm) after 20 days of inoculation. Two in one showed relatively low resistant against MoT when compare to the absolute control at 20 days after incubation. Result of the *in-vitro* test showed that Nativo and Trooper were the most effective fungicide against MoT that reduce 100% mycelial growth, followed by Azonil (82 %) and Opponent (80%) whereas the least effective to reduce colony growth of the pathogen MoT was Two in one (45%). Similar results were observed by Kabir et al. (2021) who reported that Nativo, Sunfighter, Filia and trooper completely inhibited the mycelial growth of MoT on PDA media at 20 days after inoculation.

Table 2. *In-vitro* mycelial growth inhibition of MoT by different fungicides at different days after incubation

Treatments	Mycelial growth (mm) with days				Percent Inhibition over control			
	24h	48h	72 h	20 days	24h	48h	72 h	20 days
Control	10.0 a	20.0a	25.0a	90.0 a	0.00b	0.00d	0.00e	0.00d
Nativo	0.00b	0.00d	0.00d	0.00 d	100.00a	100.00a	100.00a	100.00a
Trooper	0.00b	0.00d	0.00d	0.0 d	100.00a	100.00a	100.00a	100.00a
Two in one	0.00b	10.00b	12.00b	49.50 b	100.00a	50.00c	52.00d	45.00c
Opponent	0.00b	3.00c	4.00c	18.00 c	100.00a	85.00b	84.00c	80.00b
Azonil	0.00b	2.50cd	3.00cd	16.20 c	100.00a	87.50b	88.00b	82.00b

Note: figures in a column with different letters are significantly different.

Performance of different fungicides against wheat blast in pot experiment

In pot, overall diseases levels were very high in both experimental periods (2018-2019 and 2019-2020). Disease's symptoms appeared at approximately 14 days after inoculation in control plants as well as in fungicides treated plants (Figure 2). Both blast incidence and severity were recorded for 7 times at 2 days intervals. Wheat plants treated with different fungicides showed significantly different levels of diseases incidence. As diseases pressure was high in both cropping season as a result disease developed drastically. At 68 days age blast incidence in fungicides treated pots ranges from 15.27 – 33.02% which increased with time and reached the highest level at the age of 86 days i.e., 40.10 – 85.10 % (Table 3). In the untreated plants, blast incidence was 33.02 % at 68 days age which flared up to 85.10 % on the 86 days. Among the tested five fungicides, significantly the lowest diseases incidence was found from Nativo (40.10%) at 86 days after sowing (DAS) which was followed by Trooper (47.36%), Azonil (53.90%), Opponent (61.52 %) and two in one (69.49%). As per the performance of diseases incidence, Nativo was found the best where 52.88 % reduction of disease was observed over control at 86 DAS. As Trooper showed 44.35 % disease reduction over control at 86 DAS, so it proved as the second-best treatment.

Effect of different treatments showed significant variation on disease severity of wheat blast (Table 4). The results are presented in Table 4. On 68 DAS age, among five fungicides the highest disease severity (59.08) was found in Azonil, while the lowest disease severity was found in (26.79 %). At 86 days Nativo treated plot showed significantly lowest disease severity (91.81%). Regarding blast severity and diseases control over unsprayed pots, the application of foliar fungicide Nativo showed significantly less blast severity and better diseases control by around 91.81% and 8%, respectively followed by another fungicide treatment of Trooper (97.45 and 2% respectively) which is statistically similar to that of Azonil, Opponent, Two in one and control. Better effectiveness on grain yield was observed with Nativo. The fungicide Nativo showed the highest grain yield (2.90 t/ha), followed by treatment Azonil of 2.55 t/ha while the least grain yield observed in Control (0.24 t/ha). Roy et al. (2020) reported that among

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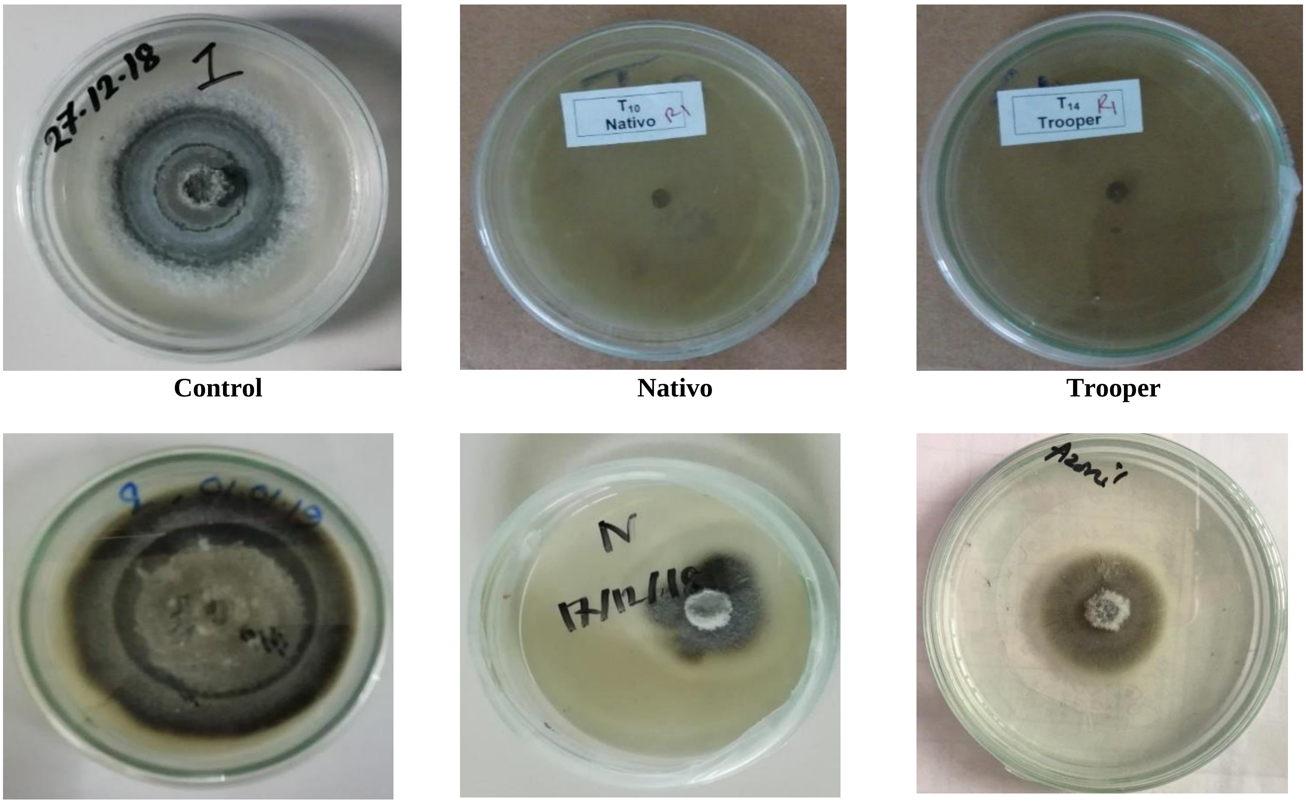
19 chemical fungicides, 2 botanicals, 1bio-agen, Nativo 75 WG, Amistar Top 325 SC and Cabrio Top 60 WG demonstrated the best results with least blast severity and the highest grain yield.



Control **Nativo** **Trooper**

Two in one **Opponent** **Azonil**

Figure 1. Growth of *Magnaporthe oryzae Triticum* on PDA poisoned with fungicides at 20 days after inoculation.

Others Treatments **Control**

Figure 2. Treated wheat plants of var. BARI Gom 26 with five different fungicides.

Control of wheat blast is a very tactful mechanical. In the present investigation control of wheat blast was not satisfactory. Its might be due to the insufficient protection given by Solo spray of fungicide at pre-heading stage or improper timing or incomplete application, poor active ingredient activity, low dose, poor coverage or extreme weather conditions during application. Some studies show that fungicides partially control wheat head blast and offer better control on cultivars with at least moderate levels of resistance (Kohli et al. 2011; Rios et al. 2016). Rocha et al. (2014) found that the control provided by fungicide applications was only effective on flag leaves, but not on

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heads. Kabir et al. (2021) found that combine 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. Cruz et al. (2019) recognized that higher diseases reduction and yield increase found by using Mancozeb based fungicides with moderate to high diseases pressure in Brasil, while in Bolivia, the combination of QoI + DMT premixes gives the best results. Rios et al. (2016) found that wheat blast can be controlled to a higher level (88–95%) using a combination of 13.3% epoxiconazole + 5% pyraclostrobin for single application. But in other studies, Pagani et al. (2014) showed that levels of control ranged from 48-72% with twice application of the same fungicide. Bockus et al. (2015) described those effective fungicides reduced MoT sporulation by 52.2 to 100% relative to a non-treated control. According to Urashima and Kato (1994), probenazole and tricyclazole gave good control of blast, except at heading stage. Another reason of getting poor performance of fungicides might be the capacity of blast pathogen to acquire resistance to fungicide extensively (Olivera et al. 2015). Rios et al. (2016) recommended combining both genetic resistance and fungicide treatment for most effective blast management. With 70 and 90% control of final incidence and severity, they found that effect of resistance and fungicide was additive of incidence as well as severity control.

Table 3. Mean Incidence (%) of wheat blast wheat cv. BARI Gom-26 treated with fungicides in the net house of BINA during 2018-2019 and 2019-2020 cropping season.

Treatments	Diseases Incidence (%)						
	68 DAS	71 DAS	74DAS	77 DAS	80 DSA	83 DAS	86 DAS
Control	33.02 a	45.75 a	54.99 a	59.20 a	61.39 a	77.52 a	85.10 a
Nativo	15.27 d	18.26 e	23.64 d	28.12 d	30.59d	36.58 d	40.10 f
Trooper	18.81 d	28.41 cd	32.61c	43.02 b	44.89 b	46.02c	47.36 e
Two in one	32.20 a	41.36 b	43.77 b	58.37a	62.71a	64.64 b	69.49 b
Opponent	24.32 c	27.35 d	31.99 c	35.65 c	40.96 c	46.11c	61.52 c
Azonil	28.10 b	30.67 c	33.00 c	35.94 c	39.220 c	45.35 c	53.90 d

Table 4. Mean Blast disease severity (%) and grain yield of wheat var. BARI Gom-26 after post-inoculation in the net house of BINA during 2018-2019 and 2019-2020 cropping season treated with five different fungicides.

Treatments	Disease severity (%)							Yield
	68 DAS	71 DAS	74DAS	77 DAS	80 DSA	83 DAS	86 DAS	(ton ha-1)
Control	72.00 a	75.39 a	84.14 a	86.85 a	96.16a	100.00a	100.00 a	0.24 e
Nativo	44.30 d	50.63c	56.50 d	65.30 e	79.85c	82.52b	91.81b	2.90 a
Trooper	26.79 e	38.12d	46.19 e	53.39 c	68.16d	84.19 b	97.45 a	1.97c
Two in one	43.05 d	48.47c	56.72 d	71.08 d	77.19c	88.62 b	98.51a	1.49 d
Opponent	51.41c	60.60 b	68.55 c	71.30 c	79.30c	86.60 b	97.29 a	1.99 c
Azonil	59.08 b	61.97 b	75.45 b	77.71b	92.75b	98.10a	99.06 a	2.55 b

Note: mean incidence is the average of six replications and numbers follows by the same letter do not differ significantly at the 5% level, DAS: Days after Sowing.

In our study, Two in one, Opponent and Azonil could not stop blast infection motion. However, Nativo and trooper brought the blast incidence and yield to a satisfactory level but fail to arrest blast severity. Some studies show Nativo found to be excellent with least wheat head blast severity (Kabir et al. 2021; Roy et al. 2020). These differences in the results may be attributed to differences in timing to anticipating sprays to target MoT, existing environmental conditions and virulence in the MoT pathogen, dynamics of MoT inoculum buildup and its sensitivity to the fungicides.

Our research indicates among tested fungicides Nativo and Trooper are equally effective in wheat blast disease control. More effective control of blast may be achieved by giving three sprays (solo or cocktail) of them (Nativo and Trooper) beginning from booting stage of wheat at three days interval.

CONCLUSION

In Bangladesh most of the tested fungicides can control the Wheat Blast disease effectively and among them, Nativo 75 WG (Tebuconazole 50% + Trifloxystrobin 25%) and Trooper 75WP (Tricyclazole) were found to be excellent with least wheat head blast severity and higher yield increase over control. Therefore, it can be recommended to use these fungicides against wheat blast with first spraying at pre-heading stage followed by a second spray at 10 days later to have effective control of Wheat Blast and higher grain yield under pot condition.

Disclosure statement

The authors have declared no conflicts of interest.

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