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PLANT PATHOLOGY | ORIGINAL ARTICLE

Effect of chemical and non-chemical management approaches in controlling wheat blast

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ABSTRACT

Wheat blast caused by *Magnaporthe oryzae* Pathotype *triticum* (*MoT*) became one of the common nuisances to the expansion of wheat production in Bangladesh. To find out a suitable control measure, fourteen chemical fungicides and five *Trichoderma asperellum* isolates were evaluated against *MoT* in *in vitro* condition. In *in vitro* conditions the efficacies of fungicides and *Trichoderma* isolates were evaluated by the measurement of percent inhibition of radial mycelial growth of *MoT*. The highest percentage of radial mycelial growth inhibition (100%) of *MoT* was obtained by Mancozeb 63% + Carbendazim 12% (Companion), Carbendazim (Autostin 50 WDG), Pyrochlostrobin 5% + Metiram 55% (Cabrio Top), Tebuconazol (Folicur EW 250) and Hexaconazol (Contaf 5EC) fungicides at low concentration (0.0125%) compared to other fungicides at 10 days after inoculation (DAI). The maximum percent of radial mycelial growth inhibition (58.24%) of *MoT* was achieved by *Trichoderma* isolate PMILTE-N51 (58.24%) followed by isolate PMILTE- N41 (53.85%) in the *in vitro* dual culture assay at 7 DAI. In pot experiment, seven fungicides (Nativo 75WG, Companion, Autostin, Indofil M45, Supreme 250EW, Contaf, Amiscore 32.5 SC) at 0.1% & 0.3% and bioagents (*T. asperellum*, PMILTE-N51-suspension, PMILTE-N51- formulation and *T. herzianum* based IPM LAB- Formulation) were used to evaluate their efficacy in controlling wheat blast disease and their effects on different yield contributing parameters of wheat. Minimum percentage of blast incidence and severity were recorded for Supreme 250 EW @ 0.3% (14.88% and 2.21%) treated plants followed by PMILTE-N51 suspension compared to control (85.06% and 90.2%). Maximum number of ear/pot (13.67), number of total and healthy spikelets/ear (55.33 and 52.33), number of total and healthy grains/ear (50.33 and 45.67) were achieved by Amiscore 32.5 SC @ 0.3% followed by Supreme 250EW @ 0.3% and a maximum weight of 1000 grains/pot (55 gm) was recorded for PMILTE-N51-suspension, whereas controlled plants showed the lowest value for all the yield contributing parameters among the treatments used in this experiment. These results indicate that Amiscore 32.5 SC and Supreme 250 EW (@ 0.3%) showed the best results as chemical control approaches, where *Trichoderma* (PMILTE-N51-suspension) can be a better non-chemical management approach against wheat blast in Bangladesh.

Keywords: Wheat blast, *Magnaporthe oryzae*, *Trichoderma*, chemical control

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1 Introduction

Wheat is a temperature-sensitive grain crop, which is the most important grain crop after rice in Bangladesh. The trend of wheat consumption is increasing day by day in this country following global trending (Mottaleb, 2020). Every year, on average 1.4 million tons of wheat is being imported in our country just only to meet the increasing demand of consumers (Ahmed, 2017). Bangladesh has imported around 6.4 MMT in the 2020-2021 year (USDA, 2020). The area under wheat crop was 3,32,274 ha in the 2019-2020 financial year which is 0.58% higher than 2018-19 (BBS, 2020) and its overall demand is increasing 13% per year in Bangladesh (BWMRI, 2020).

However, compared with the high demand, wheat production is not increasing at the same rate because of several issues. Along with wheat blast disease, wheat plants are suffered from more than 20 different diseases in Bangladesh (Fakir, 2001; Alam et al., 1994; Ahmed, 1994; Fakir et al., 1990). Among them recently the most terrible fungal disease of wheat caused by *Magnaporthe oryzae* Pathotype *triticum* (*MoT*) is recognized as blast. In February 2016, wheat blast has been spotted in Bangladesh as the first case in Asia and confirmed with genome sequencing of the causal pathogen (Islam et al., 2016). Wheat blast might arrived from South America to this country and caused up to 90 percent yield losses of more than 15000 hectares of land in the year 2016 (Islam et al., 2016). Wheat blast has some disastrous behaviour. Wheat blast disease can significantly reduce the yield and quality of susceptible wheat variety under favourable weather condition in Bangladesh. Along with favourable weather conditions, there are very few tolerant wheat varieties like BARI Gom 33 exist in Bangladesh against this disease under low disease pressure in the field. The effective field control of this seed-borne pathogen is not suitable with specific control measures.

So, until the development of the workable resistant variety, the only way for the effective control of this pathogen in the field is an integrated approach. Kohli et al. (2011) mentioned that to eliminate the seed-borne infection by *MoT*, seed treatment with fungicide can help. Pagani et al. (2014) said that to reduce the disease severity of wheat blast in the field condition spraying is needed with fungicides namely tebuconazole and tebuconazole + trifloxystrobin. In South America, mixtures of triazoles (tebuconazole and metconazole) and strobilurins have been used effectively to control wheat blast in moderate resistant wheat varieties (Kohli et al., 2011). Many fungicides are tested effective against wheat blast disease in Bangladesh but among them Tebuconazole 50% + Trifloxystrobin 25%,Azoxystrobin 20% + Difenoconazole 12.5% and Pyraclostrobin 5% + Metiram 55% containing fungicides were found to be excellent with least wheat head blast severity and higher yield increase over un-sprayed control (Roy et al., 2020). Sometimes chemical applications have not provided effective control of susceptible wheat varieties in disease-conductive environments (Goulart, 2005; Urashima, 1994; Urashima et al., 2009). As well as, the use of chemical fungicides for controlling this disease might have health hazards for human beings and animals (Zohura et al., 2018). Bio-agents like *Trichoderma* can work against fungal pathogens either directly through mechanisms such as mycoparasitism, or indirectly by competing for nutrients and space, modifying environmental conditions, promoting plant growth and plant defence mechanism and antibiosis (Shakeri and Foster, 2007; Reino et al., 2007). After following a lot of studies, it is clear that bio-control agents like *Psuedomonas fluorescens*, *Trichoderma* spp., *Bacillus subtilis*, *Bacillus methylotrophicus* and *Pseudomonas stutzeri* etc. are very effective against *Magnaporthe oryzae* Pathotype *triticum* (Cabot et al., 2018; Dutta et al., 2018; Chakraborty et al., 2020).

Therefore, this study has been designed to know the effect of 14 fungicides and *Trichoderma* spp. based bio-agents on *Magnaporthe oryzae* Pathotype *triticum* in *in vitro* conditions and to determine their effects on disease incidence and severity of the wheat blast and on yield contributing parameters of wheat in pot experiment.

2 Materials and Methods

2.1 *In vitro* experiment

2.1.1 Screening of fungicides against *MoT* isolate

Fourteen fungicides with four concentration levels were used in the i*n vitro* inhibition test (Table 1).

2.1.2 Preparation of OMA plates

For preparing oat meal agar (OMA) plates, 20 g of commercial oat meal agar powder (Himedia, India) was added to 1 L double distilled water in a volumetric flask, stirred properly and autoclaved (Akter, 2019). To prepare the 0.0125, 0.025, 0.05, and 0.1% solution of fourteen different fungicides with OMA medium, 0.125, 0.25, 0.5, and 1 g of each of them were taken separately in 1 L autoclaved OMA medium and stirred properly with the help of magnetic stirrer. Then the OMA media containing different fungicides with different concentrations were poured into petridishes for the preparation of OMA plate of the subsequent study. In this way, OMA plates supplemented with fungicides were prepared.

2.1.3 Inoculation of OMA plates with *MoT* isolate

In this study, OMA plates supplemented by different fungicides with different concentrations were inocu-

Table 1. List of fungicides along with their active ingredients used in this study

Trade name of fungicide[†]	Active ingredient
Nativo 75 WG	Tebuconazol 50% + Trifloxistrobin25%
Blitox 50 WP	Copper Oxichloride 50%
Blastin	Trifloxistrobin 25% + Tebuconazol 50%
Companion	Mancozeb 63% + Carbendazim 12%
Trooper 75 WP	Tricyclazole 75%
Indofil M 45	Mancozeb
Awal 72 WP	Zineb 68% + Hexaconazol 4%
Fiesta Z-78	Zineb
Autostin 50 WDG	Carbendazim
Metaril 72 WP	Mancozeb 64% + Metalaxyl 8%
Cabrio Top	Pyraclostrobin 5% + Metiram 55%
Bounty 36 WP	Cymoxanil 6% + Chlorothalonil 30%
Folicur EW 250)	Tebuconazol 25%
Contaf 5EC	Hexaconazol

[†] All fungicides were used @ 0.0125, 0.025, 0.05, and 0.1% as per the treatment

lated with a pure culture of *MoT* isolate PMIL*MoT*-4 collected from Plant-Microbe Interaction Laboratory, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh. Shortly, From a 10-day-old culture of *MoT*, mycelial blocks were easily excised near the edges using a sterile 5 mm diameter cork borer. These blocks were then transferred to the center of the OMA plates supplemented with different fungicides using a sterile needle. Three replications were maintained for each of the concentrations belonging to different fungicides supplemented OMA plates. Control OMA plates didn't supplement with any fungicides in the study and were inoculated with the block of *MoT* as described above.

2.1.4 Measurement of mycelial growth rate

After 10 days of inoculation, radial mycelial growth of *MoT* was recorded by measuring the average of two diameters at right angles to one another. The same procedures were repeated three times for three replications and the mean of mycelial growth was calculated. Then the efficacy of the fungicides was calculated as percent growth inhibition using the following formula reported by (Satish et al., 2007; Dubey et al., 2009).

$$\text{Growth inhibition (\%)} = \frac{C - T}{T} \times 100 \qquad (1)$$

where, C = Mean mycelial growth (radial) of pathogen in control plate and T = Mean mycelial growth (radial) of pathogen in treated plate.

2.1.5 Effect of *T. asperellum* on *MoT*

Five *Trichoderma asperellum* isolates (Isolate PMILTE-R1, Isolate PMILTE-N41, Isolate PMILTE-R50, Isolate PMILTE-HI, Isolate PMILTE-N51) collected from Plant-Microbe Interaction Laboratory, Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh were used in this study to know the antagonistic effect of *T. asperellum* against *MoT* pathogen. However, the antagonistic potential of the *T. asperellum* isolates against *MoT* was tested by dual-culture techniques (Dennis and Webster, 1971) using Potato Dextrose Agar medium. To observe the effect of *T. asperellum* on the growth of *MoT*, 7 days old cultures of *T. asperellum* isolates and 10 days old cultures of *MoT* were placed on the opposite ends of the same petridishes poured with PDA at an equal distance from the periphery on the same day. There were three replications maintained for both the treated and non-treated control plates. After that, the control plates for both the fungi and treated plates i.e. the dual cultured plates were incubated at 25 °C for 7 days. The colony diameter of radial growth of targeted fungal pathogen was measured at 3, 5, and 7 days intervals in cm and percent inhibition of average radial mycelial growth were also calculated following the procedure mentioned above.

2.2 Net house experiment

2.2.1 Experimental site and pot preparation

The pot experiment was conducted in the Professor Golam Ali Fakir Seed Pathology Centre, Bangladesh Agricultural University, Mymensingh, Bangladesh during the period from November 2019 to March 2020. BARI Gom 26 which is one of the high-yielding variety of wheat in Bangladesh and susceptible to wheat blast disease was used for the nethouse experiment. Five kg soil which was silt-loamy in nature, low to medium organic matter, pH 5.1-5.6 belonging to Old Brahmaputra Floodplain (AEZ-9) was used to fill up each of the plastic pots. In total, 54 pots

Table 2. Specification of treatments for pot experiment

Treatments	Type	Active ingredient	Concentration (%)
T1 (Nativo 75 WG)	Chemical	Tebuconazol 50% + Trifloxistrobin 25%	0.1
T2 (Nativo 75 WG)	Chemical	Tebuconazol 50% + Trifloxistrobin 25%	0.3
T3 (Companion)	Chemical	Mancozeb 63%+ Carbendazim 12%	0.1
T4 (Companion)	Chemical	Mancozeb 63%+ Carbendazim 12%	0.3
T5 (Autostin 50 WDG)	Chemical	Carbendazim	0.1
T6 (Autostin 50 WDG)	Chemical	Carbendazim	0.3
T7 (Indofil M- 45)	Chemical	Mancozeb 75%	0.1
T8 (Indofil M- 45)	Chemical	Mancozeb 75%	0.3
T9 (Supreme EW 250)	Chemical	Tebuconazole	0.1
T10 (Supreme EW 250)	Chemical	Tebuconazole	0.3
T11 (Contaf 5 EC)	Chemical	Hexaconazole	0.1
T12 (Contaf 5 EC)	Chemical	Hexaconazole	0.3
T13 (Amiscore 32.5 SC)	Chemical	Azoxystrobin 20% + Difenoconazole 12.5%	0.1
T14 (Amiscore 32.5 SC)	Chemical	Azoxystrobin 20% + Difenoconazole 12.5%	0.3
T15 (PMILTE-N51-suspension)	Biological	*Trichoderma asperellum*	$5{\times}10^{-5}$ conidia mL^{-1}
T16 (PMILTE-N51- formulation)	Biological	*Trichoderma asperellum*	3 g L^{-1}
T17 (IPM LAB- Formulation)	Biological	*Trichoderma harzianum*	3 mL L^{-1}
T0 (Control)	-	-	-

were prepared for 18 treatments including control and each of the treatments was maintained with 3 replications. Twenty (20) seeds in each pot were sown on 15 November 2020. For the maintenance of the optimum plant population, thinning was done at 30 days after sowing (DAS). Urea, MoP and TSP were applied in the pot as per requirement (BARC, 2012). Irrigation was applied from time to time when required. Thinning was done to maintain a population of fifteen plants per pot.

2.2.2 Treatments

To know the effect of fungicides and suspension as well as the formulated product of *Trichoderma* fungi on the disease incidence and severity of wheat blast and different yield contributing parameters of wheat, the following eighteen treatments including control were used in this study (Table 2). Chemical fungicides and *T. asperellum* isolate were selected based on the i*n vitro* growth inhibition results found in this experiment. IPM LAB- formulation (Biotech care, *Trichoderma harzianum* suspension, IPM lab, BAU) was also used in this study to compare the efficacy among the bioagents (Table 2).

2.2.3 Preparation of fungicide solution and *Trichoderma* suspension

0.1% and 0.3% solution of Nativo 75 WG, Companion, Autostin 50 WDG, Indofil M45 were prepared by taking 1 and 3 g from each of the above mentioned fungicides (see Table 2) in two different 1000 mL volumetric flasks and adding 1 L of distilled water in each of the flasks up to the mark of the flask, then stirring the content. On the other hand, to prepare 0.1% and 0.3% solution of Contaf 5 EC, Supreme EW 250 and Amiscore 32.5 SC, 1 and 3 mL for each fungicide were taken respectively in two different 1000 mL volumetric flasks and then required amount of distilled water was added in each of the flasks up to the mark of the flask. Suspension of *T. asperellum* isolate PMILTE-N51 was prepared by adding 10 mL sterile water into a 7-day old culture of *T. asperellum* isolate PMIL-N51 grown on PDA plate. Then the surface was scraped lightly with a sterile transfer loop and the resulting suspension was filtered through two layers of sterile muslin cloth. The conidial suspension was diluted with distilled water and made the volume 250 mL. Then the concentration of spore per mL suspension was measured with a hemocytometer and adjusted the final concentration @ $5{\times}10^{-5}$ conidia per mL. On the other hand, 3 g of PMIL-N51-formulated powder was added in 1 L volumetric flasks and 1 L of distilled water was added to make the solution. Three (3) mL IPM LAB- formulation (Biotech care, *Trichoderma* suspension, IPM lab, BAU) liquid was taken in 1 L volumetric flask and then the required amount of distilled water was added in the flasks up to the mark of the flask. After that, the content within the flask was stirred properly and IPM LAB-formulation solution was prepared for spray application.

2.2.4 Inoculation of the growing plants

For the artificial inoculation, the inoculum was prepared according to Islam et al. (2016). Briefly, the wheat plants within the pots were inoculated with a spore suspension ($5{\times}10^{-5}$ spores/mL) of *MoT* isolate PMIL*MoT*-4 at 40 DAS (just at the time of panicle emergence). After inoculation by spraying inoculum on the plants, all the inoculated pots were covered with polythene sheets keeping small holes on the sheet after inoculation. Control plants were inoculated with distilled water and covered with polythene sheets accordingly.

2.2.5 Application of different treatment solutions

Different fungicide solutions were sprayed on the plant surface as per treatments in two installments i.e. 35 and 45 DAS (five days before and after *MoT* inoculation), where *Trichoderma* suspension and formulation were sprayed at 30, 35, 45 and 50 DAS i.e. two-times spray schedules were maintained before and after *MoT* inoculation. To avoid drifting of spray materials from one pot to the neighboring ones, adequate precautions were taken.

Table 3. Disease severity scale (Maciel et al., 2013)

Grade	Description
1	No infection
2	Infection 0-3.5% on spikes
3	Infection 3.5-7.5% on spikes
4	Infection 7.5-21.5% on spikes
5	Infection 21.5-30.50% on spikes
6	Infection 30.50-44% on spikes
7	Infection 44-57.5% on spikes
8	Infection 57.5-68.0% on spikes
9	Infection 68-86% on spikes
10	Infection 100% on spikes

2.2.6 Disease incidence and severity assessment

The incidence of wheat blast disease was calculated in each pot by using the following formula (Rajput and Bartaria, 1995).

$$\text{Disease incidence} = \frac{P_I}{P_T} \times 100 \qquad (2)$$

where P_I = number of infected panicles, and P_T = number of total panicles. By observing disease symptoms on wheat spikes disease severity was obtained and assessment of severity was done by using 10 classes according to Maciel et al. (2013) (Table 3).

2.2.7 Data collection

After harvesting different data were collected on different yield contributing parameters like- plant height (cm), ear length (cm), number of ear/ pot, number of healthy and diseased ear/ pot, number of healthy and diseased grains/ear and 1000-grain weight (gm) etc. Then these collected data were analyzed.

2.2.8 Data analysis

The data of *in vitro* experiment were statistically analyzed by using the analysis of variance (ANOVA) technique to find out the level of significance (Gomez and Gomez, 1984). The effect of the treatments was compared by Duncan's Multiple Range Test (DMRT). The collected data were analyzed using SAS (University Edition Version 3.71 Basic Edition) statistical package. The pot experiment was conducted in CRD with three replications. The collected data were analyzed using SAS (University Edition version 3.71 basic edition) statistical package. The data was then statistically analyzed and tested at 5% level of significance to interpret the treatment differences Least Significant Difference (LSD) was applied.

3 Results

3.1 Effect of fungicides on the radial mycelial growth of *MoT*

Effect of fourteen fungicides at four different concentrations (0.0125%, 0.025%, 0.05% & 0.1%) on the percent growth inhibition of *MoT* was observed in the laboratory experiment. The growth of *MoT* after 10 DAI (days after inoculation) in fungicide assimilated OMA suggested a significant difference among the fungicides and their doses (Fig. 1). At 10 DAI, the untreated OMA plate had the lowest growth inhibition of *MoT*. At the lower concentration (0.0125 to 0.05%), in Blitox 50 WP (Copper Oxichloride 50%) followed by Fiesta Z-78 (Zineb) showed lowest growth inhibition while the maximum inhibitions were recorded in the OMA plates containing Companion (Mancozeb 63% + Carbendazim 12%), Autostin (Carbendazim), Cabrio Top (Pyraclostrobin 5% + Metiram 55%), Folicur EW 250 (Tebuconazol 25%) and Contaf 5EC (Hexaconazol) with values of 100% respectively followed by Awal 72 WP (Zineb 68% + Hexaconazol 4%) 85.95%, Blastin (Trifloxistrobin 25% + Tebuconazol 50%) 84.32%, Nativo 75 WG (Tebuconazol 50% + Trifloxistrobin25%) 83.78% and Trooper 75 WG (Tricyclazole 75%) 81.08% after 10 days of inoculation and 100% inhibition recorded for all the chemicals both @ 0.05% & 0.1% concentrations (Table 4).

3.2 Effect of *Trichoderma* on radial mycelial growth of *MoT*

Five aggressive *T. asperellum* isolates were used to know their effectiveness in suppressing the radial mycelial growth of *MoT* in the dual culture method up to 7 days. Each of the *T. asperellum* isolates distinctly restricted the growth of *MoT* after 3 days of inoculation in dual cultures and within five days mycelial growth of *MoT* was overlapped by the mycelial growth of different *T. asperellum* isolates.

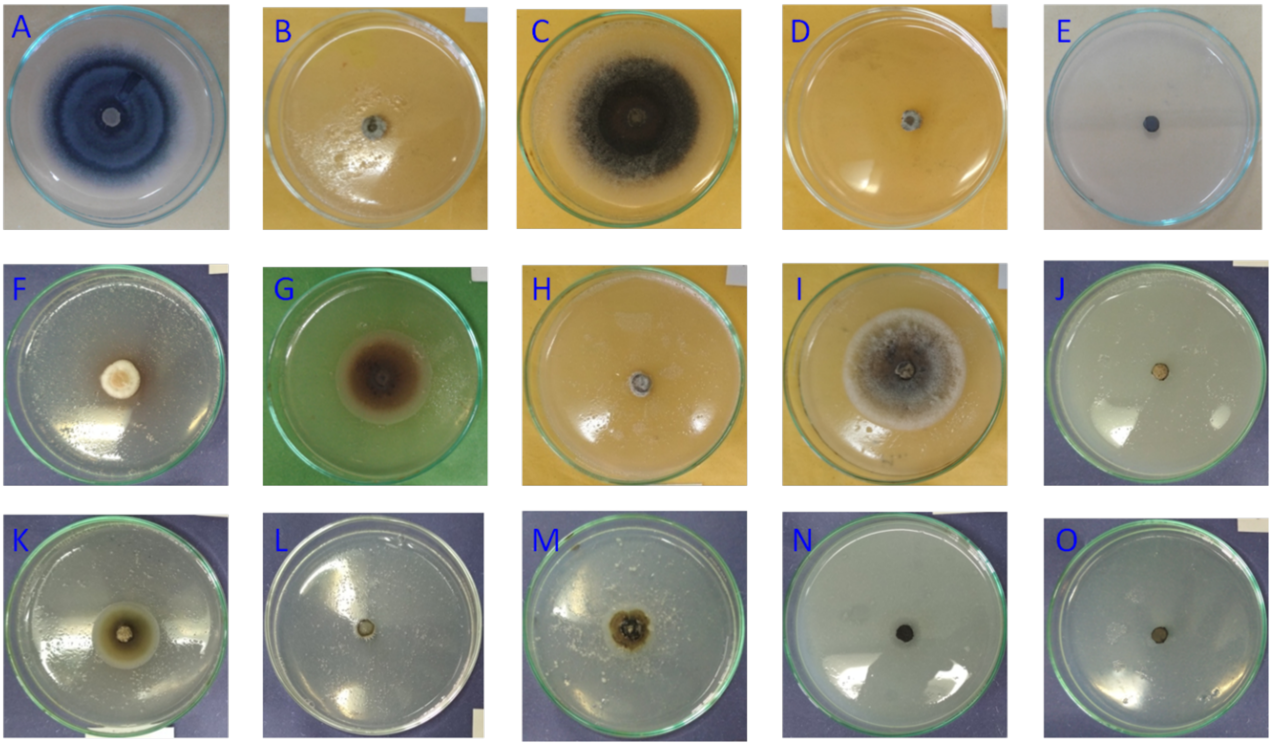

Figure 1. Status of radial mycelial growth of *Magnaporthe oryzae* Pathotype *triticum* on OMA plates at 10 DAI. Here, (A) Control and containing 0.0125% of (B) Nativo 75 WG, (C) Blitox 50WP, (D) Blastin, (E) Companion, (F) Troper 75 WP, (G) Indofil M45, (H) Awal 72 WP, (I) Fiesta Z 78, (J) Autostin 50 WDG, (K) Metaril 72 WP, (L) Cabrio Top, (M) Bounty 36 WP, (N) Folicur EW 250 and (O) Contaf 5EC.

Table 4. Radial mycelial growth and % radial mycelia growth inhibition of *MoT* over control on OMA plates containing 0.0125%, 0.025%, 0.05% and 0.1% of different fungicide(s) at 10 days after inoculation (DAI)

Fungicide	*MoT* @ 0.0125%		*MoT* @ 0.025%		*MoT* @ 0.05%		*MoT* @ 0.1%	
	RMG (cm)	RMGI (%)	RMG (cm)	RMGI (%)	RMG (cm)	RMGI (%)	RMG (cm)	RMGI (%)
Control	6.17a	-	6.17a	-	6.17a	-	6.17a	-
Nativo 75 WG	1fg	83.79	1.0e	83.79	0.00e	100	0.00f	100
Blitox 50 WP	6.11a	0.97	5.77a	6.48	4.9b	20.58	3.9b	36.79
Blastin	0.97fg	84.28	0.00f	100	0.00e	100	0.00f	100
Companion	0.00h	100	0.00f	100	0.00e	100	0.00f	100
Trooper 75 WP	1.17f	100	0.7e	88.65	0.00e	100	0.00f	100
Indofil M 45	3.7c	40.03	2.27c	63.21	1.67d	72.93	1.83c	70.34
Awal 72 WP	0.87g	85.89	0.00f	100	0.00e	100	0.00f	100
Fiesta Z-78	5.13b	16.86	4.37b	29.17	4.27c	30.79	3.6b	41.65
Autostin 50 WDG	0.00h	100	0.00f	100	0.00e	100	0.00f	100
Metaril 72 WP	2.4d	61.1	2.13cd	65.48	1.77d	71.31	1.1e	82.17
Cabrio Top	0.00h	100	0.00f	100	0.00e	100	0.00f	100
Bounty 36 WP	2.17e	64.83	1.8d	70.83	1.57d	74.55	1.4d	77.31
Folicur EW 250	0.00h	100	0.00f	100	0.00e	100	0.00f	100
Contaf 5EC	0.00h	100	0.00f	100	0.00e	100	0.00f	100
LSD(0.05)	0.23		0.43		0.28		0.15	

LSD (0.05): Least Significant Difference. In a column, means followed by same letter(s) are statistically similar at 5% level by DMRT; RMG: radial mycelial growth, and RMGI: radial mycelial growth inhibition; *MoT*: *Magnaporthe oryzae* Pathotype *triticum*

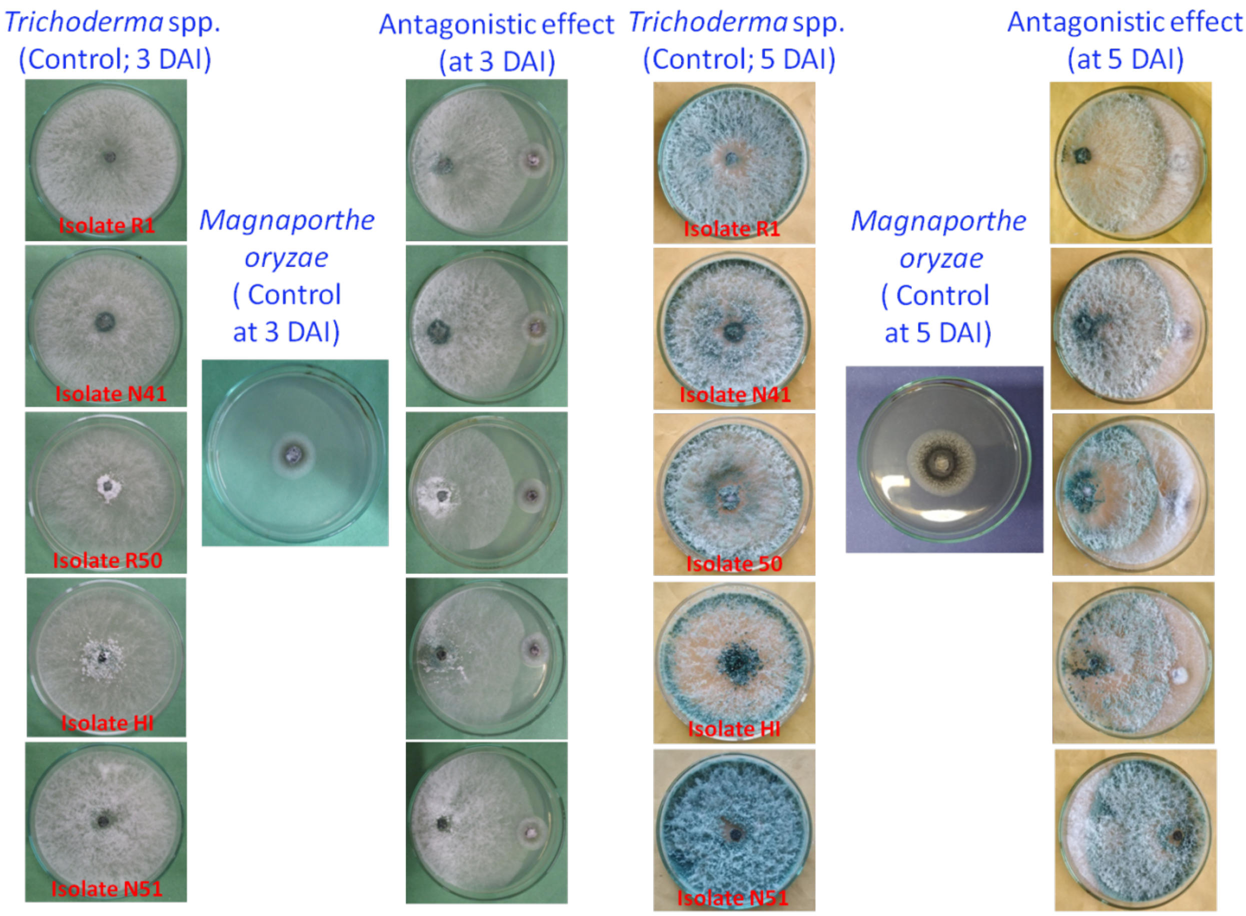

Figure 2. Comparison of radial mycelial growth of MoT in control plates and in dual cultured plates at 3rd and 5th days after inoculation (DAI)

However, radial mycelial growth of *MoT* was reduced by 49.45 to 58.24% in dual culture assays at 7 DAI. Growth suppression was the highest (58.24%) with *Trichoderma* isolate N51, followed by isolate N41 (53.85%) and isolate R50 (51.65%) shown in Table 5 and Fig. 2.

3.3 Effect of fungicides and *Trichoderma* on disease incidence and severity

Seven fungicides in two different concentrations (0.1% & 0.3%) and bio-agents (*T. asperellum* isolate PMIL N51- suspension, PMIL N51- formulation and IMP LAB- formulation a *Trichoderma harzianum* based bio-pesticide) were evaluated to know their effect on disease incidence and severity of wheat blast in the net house conditions (Table 6). Different treatments had a significant influence on percent disease incidence and severity of wheat blast at 57 DAS i.e. seven days after the second inoculation. *Trichoderma* treated plants showed better results i.e. lower disease pressure which is very much similar to the results of chemical fungicides treated plants (Table 6). However, in this experiment, the minimum percentage of disease incidence of treated plants (14.88%) was recorded for 0.3% Supreme EW 250 (T10) followed by 16.15% disease incidence for 0.3% Nativo (T2) and 17.65% disease incidence for IPM LAB-Formulation (T17) (Table 6). On the other hand, minimum percent disease severity (2.21%) was recorded for 0.3% Supreme EW 250 (T10) followed by 3.29% disease severity for PMIL-N51-suspension (T15) and 3.31% disease severity for 0.3% Amiscore 32.5 SC(T14) (Table 6). Controlled treated plants showed the maximum percent of disease incidence (85.06%) and severity (90.20%) (Table 6).

3.4 Effect of fungicides and *Trichoderma* on the yield contributing parameters

Different yield contributing parameters of wheat were also measured for some selected treatments in inoculated and control plants. The results of different yield contributing parameters such as plant height, number of ear/pot, number of healthy ear/pot and number of diseased ear/pot, number of total grains/ear, number of healthy grains/ear, number of diseased grains/ear and weight of 1000 seeds (g)

Table 5. *In vitro* mycelial growth suppression of *M. oryzae* Pathotype *triticum* with *T. asperellum* isolates

Trichoderma isolate	Days after inoculation (DAI)		
	3 DAI	5 DAI	7 DAI
RMG (cm) on control plate			
Isolate PMILTE - R1	2.35a	3.35a	4.55a
Isolate PMILTE - N41	2.35a	3.35a	4.55a
Isolate PMILTE - R50	2.35a	3.35a	4.55a
Isolate PMILTE - HI	2.35a	3.35a	4.55a
Isolate PMILTE - N51	2.35a	3.35a	4.55a
LSD (0.05)	0.8223	0.9163	0.9163
RMG (cm) on dual cultured plate			
Isolate PMILTE - R1	2.3a	2.3a	2.3a
Isolate PMILTE - N41	2.1c	2.1c	2.1c
Isolate PMILTE - R50	2.2b	2.2b	2.2b
Isolate PMILTE - HI	2.2b	2.2b	2.2b
Isolate PMILTE - N51	1.9d	1.9d	1.9d
LSD (0.05)	0.0088	0.0088	0.0088
RMGI (%) dual cultured plate over control plate			
Isolate PMILTE - R1	2.13	31.34	49.45
Isolate PMILTE - N41	10.64	37.31	53.85
Isolate PMILTE - R50	6.38	34.33	51.65
Isolate PMILTE - HI	6.38	34.33	51.65
Isolate PMILTE - N51	19.15	43.28	58.24
LSD (0.05)	-	-	-

LSD (0.05): Least Significant Difference. In a column, means followed by the same letter(s) are Statistically similar at 5% level by DMRT.

for each treatment were visibly different in inoculated and control plants after treating with different fungicides and bio-agents (Table 7). The maximum height (75.4 cm) of the wheat plant was recorded in the case of Supreme EW 250 0.3% (T10) followed by Nativo 0.3% (T2). The maximum ear length was 9.16 cm for Nativo 0.3% (T2) followed by Supreme EW 250 0.3% (T10) 9.07 cm &Autostin 0.3% (T6) 8.90 cm. In the case of the number of total, healthy and diseased ear/pot, Amiscore 32.5 SC (0.3%) treated plants showed the best results(13.67, 12.67 and 1) compared to other treatments followed by Supreme EW 250 0.3% (13.33, 11.67 and 1.67). The highest number of total grain/pot (50.33) found in Supreme EW 250 (T10) and Amiscore 32.5 SC 0.3% (T14) treated plants, the highest number of healthy grain/pot (45.67) foundin Supreme EW 250 (T10) and Amiscore 32.5 SC (T14) treated plants followed by PMIL-N51- formulation(T16) and Nativo 0.3% (T2) treated plants(46.67). On the other hand, the lowest number of diseased grain/ear was found in Nativo 0.3% (T2) treated plants (Ahmed, 2017) followed by PMIL-N51- formulation(T16) (2.33), Companion 0.3% (T4) 3.00 and Nativo 0.1% (T1) 3.33 treated plants, respectively. In case of the weight of 1000 seeds, PMIL-N51-suspension (T15) sprayed plants showed better results (55 g) followed by Supreme EW 250 0.3% (T10) 53 g and Amiscore 32.5 SC 0.3% (T14)treated plants (52.67 g) where controlled plants showed the worst value for all the yield contributing parameters (Table 7).

4 Discussion

In recent years wheat blast caused by *Magnaporthe oryzae* Pathotype *triticum* (*MoT*) have become one of the major threats to wheat production which may cause yield losses up to 15-100% (Peng et al., 2011; Islam et al., 2016). Now-a-days, wheat blast has reached 20 different districts of Bangladesh and becomes a usual disease of wheat in Bangladesh especially in the southeast region of the country. So, proper management of wheat blast is very important for the sustainable production of wheat in Bangladesh. Therefore, a more convenient, rapid and cost-effective management practice should need to be developed for the management of this destructive wheat disease.

In this study, the results of the laboratory experiment distinctly showed that Companion (Mancozeb 63% + carbendazim 12%), Autostin 50 WDG (Carben-

Table 6. Effect of different fungicides and bio-agents treatments on wheat blast incidence and severity

Treatments	% Disease incidence	% Disease severity
T1 (Nativo 0.1%)	28.10 ef	15.86 d
T2 (Nativo 0.3%)	16.15 h	3.47 g
T3 (Companion 0.1%)	38.20 cd	5.42 fg
T4 (Companion 0.3%)	30.97 e	9.49 ef
T5 (Autostin 0.1%)	39.32 c	27.23 c
T6 (Autostin 0.3%)	29.66 ef	11.37 de
T7 (Indofil M 0.1%)	28.99 ef	4.01 g
T8 (Indofil M 0.3%)	19.64 gh	3.79 g
T9 (Supreme EW 250 0.1%)	24.07 fg	4.79 g
T10 (Supreme EW 250 0.3%)	14.88 gh	2.21 g
T11 (Contaf 0.1%)	32.75 de	13.36 de
T12 (Contaf 0.3%)	34.11 cde	12.14 de
T13 (Amiscore 32.5 SC 0.1%)	58.66 b	49.57 b
T14 (Amiscore 32.5 SC 0.3%)	20.35 gh	3.31 g
T15 (PMILTE-N51-suspension)	28.58 ef	3.29 g
T16 (PMILTE-N51- formulation)	27.69 ef	3.67 g
T17 (IPM LAB- Formulation)	17.65 h	3.51 g
T0 (Control)	85.06 a	90.20 a
LSD(0.05)*	6.5046	4.512

LSD (0.05): Least Significant Difference. In a column, means followed by the same letter(s) are Statistically similar at 5% level by DMRT.

dazim), Cabrio Top (Pyrochlostrobin 5% + Metiram 55%), Folicur EW 250 (Tebuconazol 25%) and Contaf 5EC (Hexaconazol) fungicides followed by Awal 72 WP (Zieneb 68% + Hexaconazol 4%), Blastin (Trifloxistrobin 25% + Tebuconazol 50%), Nativo 75 WG (Tebuconazol 5% + Trifloxistrobin 2.5%) and Trooper 75 WP (Tricyclazole 75%) were found highly effective in low concentration (0.0125%) i.e. they inhibited mycelial growth up to 100 percent at 0.0125% compared to other fungicides used in this study. So it was found that Tebuconazol, Carbendazim, Tricyclazole, Hexaconazol containing fungicides and their combination with other fungicides were more effective to control *MoT* compared to Copper Oxichloride 50%, Zineb, Mancozeb, Mancozeb 64% + Metalaxyl 8%, Cymoxanil 6% + Chlorothalonil 30% containing fungicides. On the other hand, growth suppression of *MoT* was the highest (58.24%) with Trichoderma asperellum isolate N51, followed by isolate N41 (53.85%) and isolate R50 (51.65%). These findings are in agreement with the findings reported by other researchers. Islam et al. (2016) stated that spraying with fungicides namely tebuconazol and tebuconazole + trifloxystrobin can reduce the disease severity of wheat blast in the field condition. In South America, mixtures of triazoles (tebuconazole and metconazole) and strobilurins have been used effectively to control head blast in moderate resistant wheat varieties (Islam et al., 2016). According to Urashima (1994), probenazole and tricyclazole gave good control of blast, except at the heading stage. Some combinations of earlier reported fungicides *viz*., tricyclazole and tebuconazole were reported (Goulart and Paiva, 1990) to give the best yield increase if followed by thiophanate-methyl+ mancozeb. Roy et al. (2020) evaluated the performance of different fungicides againstwheat blast under field conditions and found that among the fungicides Tebuconazole 50% +Trifloxystrobin 25%, Azoxystrobin 20% + Difenoconazole 12.5% and Pyraclostrobin 5% + Metiram 55% showed the best results with least blast severity (4–8%) and the highest yield (78–88%). Some beneficial microorganisms such as bacterial strains of *Pseudomonas* spp., *Bacillus* spp., (Gnanamanickam and Mew, 1992), *Streptomyces* spp. (Law et al., 2017), and fungi such as *Trichoderma harzianum* (Singh et al., 2012) were found effective against rice blast and, therefore, hold promise against wheat blast (Singh et al., 2021).

On the other hand, the results of this pot experiment indicate that the minimum disease incidence and severity percentage of treated plants were recorded for Supreme EW 250 (0.3%) 2.22%and 14.88% followed by PMIL-N51-suspension (3.39% and 28.58%) and Nativo 0.3% (3.47% and 1615%), respectively and maximum percentage of disease incidence and severity were recorded in case of control plants (85.06 and 90.2%). However, these findings are in corroborate with the findings reported by other researchers. Rios et al. (2016) suggested that wheat blast can be managed effectively by using both genetic resistance and fungicide (epoxiconazole + pyraclostrobin) treatment and disease incidence and sever-

Table 7. Efficacy of foliar spray of different treatments on various yield contributing parameters of wheat

Treatments	Plant height (cm)	Ear length (cm)	No. of total ears/pot
T1 (Nativo 0.1%)	72.33 abcd	8.13 cde	11.33 bcd
T2 (Nativo 0.3%)	73.50 abc	9.17 a	12.67 abc
T3 (Companion 0.1%)	70.83 bcd	8.23 cd	10.33 d
T4 (Companion 0.3%)	70.07 cd	7.80 de	11.67 abcd
T5 (Autostin 0.1%)	71.30 bcd	8.30 bcd	7.67 e
T6 (Autostin 0.3%)	72.23 abcd	8.90 abc	10.67 cd
T7 (Indofil M 0.1%)	71.47 bcd	7.767 de	11.67 abcd
T8 (Indofil M 0.3%)	70.33 bcd	7.77 de	11.00 cd
T9 (Supreme EW 250 0.1%)	73.00 abc	8.33 bcd	11.333bcde
T10 (Supreme EW 250 0.3%)	75.40 a	9.07 ab	13.33 ab
T11 (Contaf 0.1%)	71.50 bcd	8.10 de	10.33 d
T12 (Contaf 0.3%)	71.33 bcd	7.43 e	11.67 abcd
T13 (Amiscore 32.5 SC 0.1%)	71.67 bcd	8.27 cd	11.67 abcd
T14 (Amiscore 32.5 SC 0.3%)	73.67 ab	8.90 abc	13.67 a
T15 (PMILTE-N51-suspension)	72.50 abcd	8.90 abc	12.00 abcd
T16 (PMILTE-N51- formulation)	73.33 abc	8.07 de	12.67 abc
T17 (IPM LAB- Formulation)	72.67 abc	7.70 de	11.33 bcd
T0 (Control)	69.17 d	7.57 de	7.67 e
LSD(0.05)	3.4452	0.7891	2.0861

	No. of the healthy ears/pot	No. of diseased ears/pot	No. of total grains/ear
T1 (Nativo 0.1%)	9.33def	2.00 bcde	36.33 cd
T2 (Nativo 0.3%)	11.00 bc	1.67 cde	46.67 ab
T3 (Companion 0.1%)	7.67 g	2.67 bcd	37.33 cd
T4 (Companion 0.3%)	8.00 fg	3.33 ab	36.67 cd
T5 (Autostin 0.1%)	5.67 h	2.00 bcde	34.00 de
T6 (Autostin 0.3%)	8.00 fg	2.67 bcd	37.67 cd
T7 (Indofil M 0.1%)	9.33 def	2.33 bcde	28.67 e
T8 (Indofil M 0.3%)	8.00 fg	3.00 bc	36.00 cd
T9 (Supreme EW 250 0.1%)	9.67 cde	1.67 cde	41.33 bc
T10 (Supreme EW 250 0.3%)	11.67 ab	1.67 cde	50.33 a
T11 (Contaf 0.1%)	8.33efg	2.00 bcde	37.00 cd
T12 (Contaf 0.3%)	9.667 cde	2.00 bcde	34.00 de
T13 (Amiscore 32.5 SC 0.1%)	10.00 cd	1.67 cde	36.33 cd
T14 (Amiscore 32.5 SC 0.3%)	12.67 a	1.00 e	50.33 a
T15 (PMILTE-N51-suspension)	10.67 bcd	1.33 de	45.67 ab
T16 (PMILTE-N51- formulation)	10.67 bcd	2.00 bcde	47.67 a
T17 (IPM LAB- Formulation)	10.00 cd	1.33 de	40.00 c
T0 (Control)	3.00 i	4.67 a	19.67 f
LSD(0.05)	1.6255	1.5844	5.5713

	No. of healthy grains/ear	No. diseased grains/ear	Wt. of 1000 seeds (g)
T1 (Nativo 0.1%)	33.00 def	3.33 def	31.67 ef
T2 (Nativo 0.3%)	44.67 ab	2.00 f	41.33 bc
T3 (Companion 0.1%)	30.33 efg	7.00 bc	32.67 ef
T4 (Companion 0.3%)	33.67 def	3.00 ef	35.67 de
T5 (Autostin 0.1%)	27.33 gh	6.67 bcd	37.67 cd
T6 (Autostin 0.3%)	29.67 fg	8.00 b	38.33 cd
T7 (Indofil M 0.1%)	24.33 h	4.33 cdef	41.00 bc
T8 (Indofil M 0.3%)	32.00 defg	4.00 cdef	41.33 bc
T9 (Supreme EW 250 0.1%)	36.33 cd	5.00 bcdef	44.33 b
T10 (Supreme EW 250 0.3%)	45.67 a	4.67 bcdef	53.00 a
T11 (Contaf 0.1%)	30.33 efg	6.00 bcde	43.00 b
T12 (Contaf 0.3%)	29.67 fg	4.33 cdef	42.67 b
T13 (Amiscore 32.5 SC 0.1%)	31.00 efg	5.33 bcdef	30.33 f
T14 (Amiscore 32.5 SC 0.3%)	45.67 a	5.00 bcdef	52.67 a
T15 (PMILTE-N51-suspension)	40.67 bc	5.00 bcdef	55.00 a
T16 (PMILTE-N51- formulation)	45.33 ab	2.33 f	52.33 a
T17 (IPM LAB- Formulation)	35.00 de	5.00 bcdef	32.67 ef
T0 (Control)	4.33 i	15.33 a	21.67 g
LSD(0.05)	4.9798	3.49	4.0095

LSD (0.05): Least Significant Difference. In a column, means followed by the same letter(s) are Statistically similar at 5% level by DMRT.

ity were controlled effectively by control of final incidence and severity at 70 % and 90%. Pagani et al. (2014) reported that Trifloxystrobin or Tebuconazole are recommended for controlling wheat blast disease in Bangladesh.

This research work has also presented that the different yield contributing parameters such as plant height, ear length, number of ear/pot, number of healthy and diseased ear/pot of wheat showed clear differences in inoculated and control plants after treating with different treatments (Table 6). The maximum height (75.40 cm) of the wheat plant was recorded for Supreme EW 250 (0.3%) and the maximum ear length (9.17 cm) was recorded for Nativo 0.3%. In case of the number of total, healthy and diseased ear/pot, Amiscore 32.5 SC (0.3%) (13.67, 12.67 and 1) treated plants tested with best results compared to other treatments followed by Supreme EW 250 0.3% (13.33, 11.67 and 1.67) and control plants (7, 3 and 4.67) showed the lowest results. In this experiment, it was visible that there was a distinguishable difference among the treatments in case of the number of total, healthy and diseased grains/ear of wheat. Amiscore 32.5 SC showed the best results for total and healthy grains/pot (50.33 and 45.67) whereas controlled plants showed the worst results (19.67 and 4.33). On the other hand, the weight of 1000 seeds ranged from 55 to 21.67 gm for PMIL-N51-suspension and control respectively. These findings are also supported by the findings of various researchers. Tatineni et al. (2009) showed that up to 42% yield loss was prevented by applying foliar fungicides to winter wheat. Some of the researchers suggested that some bio-control agent's *viz. Bacillus methylotrophicus*, *Chaetomium globosum* and *Trichoderma harzianum* significantly control rice blast disease (Singh et al., 2021; Park et al., 2005; de Oliveira et al., 2015). Based on the entire findings of the present research it may be concluded that Amiscore 32.5 SC (Azoxystrobin 20%+ Difenoconazole 12.5%) and Supreme 250 EW (Tebuconazole) both were most effective under field condition because they both exhibited minimum disease incidence and severity and highest yield contributing parameters at 0.3% solution. *Trichoderma*-treated plants were also tested with better results which are similar to that of chemical fungicide-treated plants.

5 Conclusion

Based on the findings of the present study it may be concluded that both Supreme 250 EW (Tebuconazole) and Amiscore 32.5 SC (Azoxystrobin 20%+ Difenoconazole 12.5%) @ 0.3% are effective for control of wheat blast disease as they showed the minimum percentage of disease incidence and severity and highest yield contributions under net house condition in the pots. Also, Trichoderma asperellum suspension can be used as they also showed good performance to manage disease incidence and severity percentage with better yield contributions. However, more research work should be conducted in the field to find out the field efficacy of these chemicals with the lowest environmental hazards and bio-control agents with different concentrations and frequencies in controlling blast of wheat with the best results.

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Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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