OVERWINTERING OF WHEAT BLAST IN BANGLADESH: EVIDENCE OF STUBBLE-BORNE PERSISTENCE WITHOUT ALTERNATIVE HOSTS

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Abstract. The fungal disease wheat blast, caused by *Magnaporthe oryzae* pathotype *Triticum* (MoT), threatens wheat production in Bangladesh and other South Asian countries, causing substantial yield losses. Identifying its overwintering mechanisms and potential alternative hosts is crucial but information on these aspects remains limited under South Asian conditions. The study investigated potential alternative hosts and assess pathogen survivability in wheat stubble. One study examined five common grass weed species, namely jungle rice (*Echinochloa colona*), barnyard grass (*Echinochloa crus-galli*), goose grass (*Eleusine indica*), crab grass (*Digitaria sanguinalis*), and Bermuda grass (*Cynodon dactylon*), along with cereal crops rice (*Oryza sativa*) and maize (*Zea mays*) in controlled conditions. A second experiment examined the survivability of the wheat blast pathogen in blast-infected wheat stubble in the laboratory. PCR analysis using the MoT-specific marker MoT3 confirmed infection only in the susceptible wheat cultivar BARI Gom 26, which developed typical blast symptoms with 84% disease severity and produced the expected 361-bp amplicon, whereas no PCR amplification or blast symptoms were detected in the tested grass weed species, rice, or maize. MoT remained detectable in infected wheat stubble for up to six months post-harvest; however, spore abundance progressively declined, and no spores were detected after seven months. While the five grass weed species, rice, and maize were evaluated in the study, they are unlikely to serve as alternative hosts for MoT under the conditions of this study. However, MoT can persist in wheat stubble for up to six months after harvesting. These findings are critical for managing wheat blast effectively in Bangladesh and other wheat-growing regions with similar agroecological conditions.

Keywords: alternate host; *Magnaporthe oryzae* pathotype *Triticum*; overwintering; stubble; survival rate; wheat blast.

INTRODUCTION

Wheat (*Triticum aestivum* L.) is the world's most widely grown cereal crop, cultivated on almost every continent because of its broad adaptation to diverse environmental and climatic conditions [21]. More than one third of the world's population consumes it as a staple food, and it accounts for 20% of global caloric intake [4]. Wheat is the second most important staple food after rice in Bangladesh and is mainly cultivated in the northwestern and southwestern regions of the country, particularly in Rangpur, Dinajpur, Meherpur, Rajshahi, Chuadanga, Jhenaidah, and Faridpur, where the winter season is relatively longer and more favourable for wheat cultivation. During the 2024–25 season, wheat was cultivated on 2.8 lakh hectares in Bangladesh, producing about 10.41 lakh tonnes, the lowest production in five years, according to the Bangladesh Bureau of Statistics (BBS). Compared with the previous year, both acreage and production declined by roughly 11% [3]. The country's annual wheat demand is around 75 lakh tonnes, of which only about 14 percent is met through domestic production, with the rest supplied via imports [3]. Wheat production in Bangladesh faces several abiotic and biotic challenges. Among the abiotic stresses, heat, salinity, and drought are the most common, whereas diseases constitute the major biotic constraint. The environmental conditions in Bangladesh are highly favourable for several wheat diseases, including leaf blight, leaf rust, Fusarium head blight, and wheat blast, the most devastating disease of wheat [24].

Wheat blast caused by *Magnaporthe oryzae* pathotype *Triticum* (MoT) is a fungal disease and has become a major threat to wheat production in South Asia [11, 16]. MoT was first discovered in Brazil in 1985 [15] and subsequently spread across central and southern Brazil, as well as parts of Bolivia, Paraguay, and Argentina [28, 32]. However, in 2016, wheat blast was reported outside South America for the first time, with outbreaks occurring in Bangladesh, particularly in districts adjacent to the Indian border [24]. In the 2016 wheat growing season, blast affected approximately 15,000 ha (3.5% of the country's total wheat area) in Bangladesh, causing yield losses ranging from 5% to 100% [16]. Furthermore, it has been estimated that approximately 60% of the wheat-growing areas in South Asia are climatically suitable for wheat blast should the disease spread to other countries in the region [17, 27].

Due to its multiple survival mechanisms (infected seed, alternative hosts, crop residues, and airborne conidia), rapid spread, spike infection, potential for fungicide resistance, high mutation rate, and the limited availability of resistant wheat cultivars, the disease is classified as one of the most destructive diseases of wheat, capable of causing yield losses of up to 100%. Although MoT can infect all above-ground parts of the wheat plant, the most severe infection occurs on the spikes during heading [11]. Common symptoms include: (i) elliptical, eye-shaped lesions on wheat leaves with white to grey centres and dark brown margins; (ii) premature bleaching of the spike above the point of infection; (iii) bleached spikes with rapid discoloration and shrivelled spikelets. Disease outbreaks are highly dependent on weather conditions, with high

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night temperatures (>17 °C), cloudy weather, and intermittent drizzle favouring wheat blast epidemics [26]. Wheat is grown in Bangladesh from November to April, followed by jute, *aus* rice or maize from April to July, and *aman* rice from July to November. Following wheat harvest, fields remain free of wheat crops for approximately seven months. This raises an important epidemiological question: how does the wheat blast pathogen survive during this wheat-free period and initiate infection in the following wheat-growing season? Some reported wheat blast alternative hosts including *Brachiaria distachya*, *B. mutica*, *Dactyloctenium aegyptium*, *Digitaria ciliaris*, *Echinochloa colona*, *Eleusine coracana*, *E. indica*, have been reported in Brazil and Bolivia [23]. MoT can infect these hosts and survive on crop residues, producing spores between successive wheat-growing seasons [31]. In addition, invasive grasses have been reported to act as epidemiological bridges, serving as sources of secondary inoculum between wheat crops [5]. However, reports on alternative hosts are not always consistent, as host susceptibility may vary depending on pathogen populations, host genotype, and environmental conditions. Consequently, it remains unclear whether the alternative hosts identified in South America play a similar epidemiological role under the agroecological conditions of Bangladesh, where wheat is cultivated in a distinct cropping system and climate. Although wheat stubble, grass weeds surrounding wheat fields, and other Poaceae crops have been proposed as potential reservoirs of MoT during the wheat-free period, experimental evidence supporting their role in Bangladesh is still limited [10]. Identifying overwintering sites and alternative hosts helps elucidate the complete life cycle of the fungal pathogen [1]. This knowledge is crucial for developing targeted and effective control strategies. Understanding where the pathogen survives during the off-season can facilitate the development of cultural practices that interrupt the disease cycle, reduce primary inoculum, and minimise disease spread in subsequent wheat crops.

Rice is the major crop in Bangladesh and is cultivated throughout the year. The blast is one of the major diseases of rice [29]. Except for head blast, the symptoms of wheat blast differ from those of rice blast [14]. Several grass weed species, including *Echinochloa crus-galli*, *Echinochloa colona*, *Digitaria sanguinalis*, *Eleusine indica*, and *Cynodon dactylon* are commonly found within and around wheat fields in Bangladesh. Wheat blast is becoming a great threat to wheat cultivation in Bangladesh and negatively affects food security. Once wheat spikelets are infected by wheat blast, effective control measures become very limited. To combat this disease, numerous initiatives have been undertaken, including the development of tolerant or resistant varieties through breeding programs, quarantine measures, cultural practices, fungicidal applications, disease forecasting, and biological control [18-20, 34]. However, systematic studies evaluating alternative hosts of wheat blast in the South Asian region remain limited. Based on previous reports, we hypothesised that selected grass weed species, cereal crops belonging to the Poaceae family, and wheat stubble could contribute to the survival of MoT during the off-season in Bangladesh.

Therefore, the objectives of this study were to (i) identify potential alternative hosts of MoT among common grass weed species and cereal crops belonging to the Poaceae family in Bangladesh, and (ii) evaluate the survival of the wheat blast pathogen in infected wheat stubble following wheat harvest.

MATERIAL AND METHODS

Experimental site

Two experiments (one screen house and one laboratory) on exploring the potential alternative host of the blast pathogen during overwintering and survivability of the pathogen in wheat stubbles after the harvest of wheat were conducted at the Department of Plant Pathology of Bangladesh Agricultural University (BAU) and Plant Pathology and Biotechnology Division of Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh. The experiments were performed during the period from October 2019 to October 2020. The experimental site has the subtropical climate characterized by high rainfall from June to September and scanty rainfall from October to April, high temperature and relatively long days (up to 14 hours) from April to September, and, low temperature and a short-day period during October to March.

Alternative host evaluation (Experiment 1)

A screen house experiment was conducted at BINA, using potted soil to investigate potential alternative hosts of MoT. Plastic pots (30 cm in height and 30 cm in diameter) were used in the trial [9]. Sandy loam soil was collected from the BINA research field. After collection, the soil was sun-dried for several days, ground to break up clods, and screened through a 10 mesh sieve to remove large particles and plant debris. The soil was then treated with 40% formalin solution at a rate of 200 mL cft^{-1} soil and kept covered with polythene sheets for three days. The soil was then uncovered and kept for two days to allow the formalin to dissipate. Well-decomposed cow dung was added to the soil at the rate of 10 t ha^{-1} and then TSP, MoP, Gypsum, Mono $ZnSO_4$ and Boric acid were mixed thoroughly with the soil at the rate of 180, 120, 5.5, 24, 12 kg ha^{-1}, respectively [2]. The prepared soil was added to each plastic pot at a rate of 15 kg soil per pot.

Healthy, mature seeds of the blast-susceptible wheat cultivar BARI Gom 26, rice cultivar BRRI dhan28, and a local maize variety were collected from BINA. Rice and maize were included because they are major cereal crops grown during the wheat-free period, whereas the five grass weed species were selected because they are commonly found within or around wheat fields in Bangladesh and belong to the Poaceae family. The species selection followed the rationale outlined in the Introduction: the selected grass weeds are common in

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wheat-growing environments, and several, particularly species belonging to the genera *Echinochloa*, *Eleusine*, and *Digitaria*, have previously been reported as potential hosts of wheat blast pathogens in South America [13, 23, 25]. Mature seeds of selected grass weed species, namely jungle rice (*Echinochloa colona*), barnyard grass (*Echinochloa crus-galli*), goose grass (*Eleusine indica*), crab grass (*Digitaria sanguinalis*), and Bermuda grass (*Cynodon dactylon*), were collected from the BAU campus during the wheat growing season. Before the experiment, seeds of the three crops (rice, wheat, and maize) and five weed species were subjected to germination tests, and satisfactory germination was observed. The seeds of each crop and weed species were sown separately in individual pots with a total of 10 seeds sown per pot at an appropriate spacing. The experimental pots were regularly monitored and protected from disturbance by animals, including birds and other potential pests. The pots were watered as required, and any naturally emerging weeds were removed. Urea fertilizer was applied in three equal splits: (a) one-third during soil preparation; (b) one-third after four weeks of sowing; (c) the remaining one-third seven weeks after sowing. Plant growth and health were monitored regularly throughout the experiment. The experiment was arranged in a completely randomized design (CRD) with four replications.

Blast-infected wheat spikes were collected from farmers' fields in Chandbill, Meherpur Sadar, Bangladesh. In the laboratory, the MoT inoculum was prepared on oatmeal agar (OMA) following the procedure of Harun-Or-Rashid *et al.* [11]. The spore suspension was adjusted to a concentration of 1×10^6 spores mL^{-1} using a hemocytometer. The MoT suspension was inoculated onto the foliar surfaces of wheat, rice, maize, and the selected weed species in a growth chamber using an atomizer. The plants were covered with a polythene sheet for 48 h to maintain high humidity and facilitate infection. The growth chamber was maintained at a mean temperature of 25 °C and 90% relative humidity with a 12-h light/12-h dark photoperiod. Before inoculation, the viability of the MoT spores was confirmed by observing spore germination in a water droplet under a compound microscope. Germ tubes were observed emerging from both ends and from the lateral cells of the spores (Fig. 1), confirming that the inoculum was viable before plant inoculation.

Disease incidence and severity were assessed on wheat, rice, maize, and the five grass weed species at 7 days after inoculation. For each replicate, ten spikes (for wheat) or the corresponding number of tillers/panicles (for rice, maize, and the grass weeds) were randomly tagged prior to inoculation and monitored individually. Disease incidence was calculated as the percentage of tagged spikes/tillers showing visible blast symptoms out of the total number tagged, using the formula: Incidence (%) = (Number of infected spikes / Total number of tagged spikes) × 100.

Disease severity was assessed at the spikelet level on each symptomatic spike. The total number of spikelets and the number of visibly infected spikelets (showing characteristic bleaching, discoloration, or shriveling) were counted on each tagged spike. Spike-level severity was calculated as: Spike severity (%) = (Number of infected spikelets / Total number of spikelets on that spike) × 100. Mean severity per replicate was obtained by averaging spike-level severity values across the ten tagged spikes, and overall severity for each host species was calculated as the mean of the four replicate values.

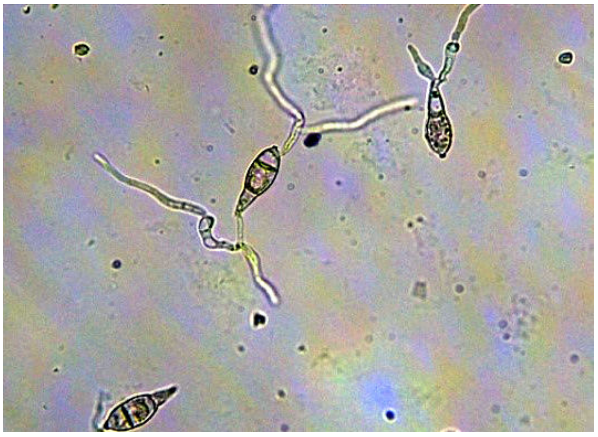

Figure 1. Germination of MoT spores observed under a compound microscope using watch glass (40 ×)

Molecular techniques

Extraction of genomic DNA from fungal isolates: A modified version of the International Rice Research Institute's (IRRI) mini-prep DNA extraction method was used. This method's PCR analysis was performed in the Biotechnology Lab of BINA using a streamLined mini-scale process for DNA isolation. For PCR analysis, the quality of the extracted DNA used in the technique was sufficient (Table 1). To gather DNA, a small pestle and mortar was used to grind a few spike samples from wheat plants, leaves from weeds, maize, and rice. During the autoclaving-based DNA extraction, strict cleanliness was followed. 1 cm × 5 cm leaf sample/spike/stubbles were cut and the sample was ground in a mortar pestle and then added 800 µL extraction buffer. For 20 minutes, the tube was heated to 65 °C in water. The tubes received 800 µL of a chloroform combination (24:1) (chloroform + isoamyl alcohol). For three minutes, the test tubes were repeatedly inverted. The materials were then centrifuged at 11000 rpm for 8 minutes. The 500 µL aqueous portion was taken out of the 1.5 mL test tube. A total of 1000 µL of cold, 100% ethanol were added, then blended by inversion. The samples were centrifuged at 13200 rpm for 12 minutes. Total 1000 µL of cold, 70% ethanol were added. All of the samples were centrifuged at 13200 rpm for 3 minutes. By inverting the test tube over on a bench for 30 to 45 minutes, the pellet was air-dried. The pellet was redissolved by warming in a 65 °C water bath for one hour after being resuspended in 100 µL of TE buffer. For future research, the DNA stock solutions were kept at 4 °C.

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Mean DNA concentrations varied across species, ranging from 228.9 ng/µL in Bermuda grass to 1242.4 ng/µL in barnyard grass (Table 1). Mean 260/280 absorbance ratios for most species fell within the 1.8–2.1 range generally indicative of good-quality DNA with minimal protein contamination, with the exception of maize (1.21) and crab grass (1.40), which showed comparatively lower purity. Mean 260/230 ratios were more variable, with crab grass (0.83), wheat stubble (1.49), and wheat spike (1.24) falling below the ideal 2.0–2.2 range, likely reflecting residual polysaccharide or phenolic compounds common in fibrous plant tissue extracts. Despite this variability, all DNA samples successfully amplified in downstream PCR reactions, indicating that extraction quality was sufficient for reliable molecular detection regardless of minor differences in absorbance ratios.

PCR and gel electrophoresis: A 10 µL PCR reaction mixture was prepared that included DNA was added to the PCR tubes and ran through the DNA thermal cycler [30]. Green Taq polymerase, 0.5 µL dNTPs, 0.25 µL DNA polymerase, 1 µL primer (MoT3 forward GTCGTCATCAACGTGACCAG and reverse ACTTGACCCAAGCCTCGAAT), and 3.75 µL ddH2O were needed for a single reaction. For the first 15 reactions, a 1.5 mL PCR tube was filled with 3.75 µL of sterilized ddH_2O, 2.5 µL of DNA polymerase, and 0.5 µL of dNTPs. Primer (forward and reverse) was then added in a volume of 1 µL.

After that, the mixture was vortexed. Finally, it was combined with 3.5 µL of Taq DNA polymerase. For PCR amplification, 1.0 µL of each template DNA sample was pipetted into the wells of the PCR tubes. The amplification process involved 30 cycles of 94 °C for 30 s, 62 °C for 30 s, and 72 °C for 60 s, followed by a final extension at 72 °C for 2 min. On 2% agarose gels, amplified PCR products were examined and stained with ethidium bromide. Each PCR product used around 2 µL of space in each well. For size assessment, a 100 bp DNA ladder was utilized as a DNA size marker. After staining, the gel was carefully removed from the staining tray and set up on a GEL Doc system with high-performance UV light box (UV-trans-illuminator) to look for DNA bands.

The MoT3 primer pair was designed by Pieck et al. [30] to amplify a genomic marker (WB12) associated specifically with the *Triticum* pathotype of *M. oryzae*, distinguishing it from other host-adapted pathotypes of the species under standard annealing conditions. While MoT3 has been widely used for MoT identification, Gupta et al. [8] reported that the marker can show reduced but detectable cross-amplification in *M. oryzae* isolates from rice under certain conditions, indicating that PCR detection alone should not be interpreted as absolute confirmation of pathotype identity. In the present study, MoT3-based detection was therefore corroborated with phenotypic disease assessment (incidence and severity scoring) on each inoculated species, providing an independent line of evidence to support host-range conclusions.

Survivability of blast pathogen in wheat stubble after harvesting of wheat (Experiment 2)

This study aimed to determine the extent of survivability of wheat blast pathogen in wheat stubbles. Immediately after the wheat harvest, blast-affected wheat stubbles were collected from the fields in Meherpur, Bangladesh. Collected stubbles were stored in the BAU laboratory in similar field conditions maintaining field temperature. Immediately after the collection of stubbles, some portions (composite samples) were used for the MoT isolation and morphological identification test and for this the stubbles were cut into pieces of 5-6 cm in length and placed in both moist chamber and agar plates. To create the moist chamber, two blotting papers (Whatman no. 4 filter paper, size 9 cm) were soaked in uncontaminated water with plastic Petri dishes. Three to four pieces were positioned in the wet chamber with equal spacing between them. At 25 ± 1 °C, the moist chambers were incubated. The incubated samples were observed under a microscope in a regular basis. From the remaining stubbles, the MoT test was done at each month's intervals starting from March 2020 to October 2020.

To perform the agar plate technique surface disinfection was performed in 10% Clorox (sodium hypochlorite solution) for 1-2 minutes, followed by 2-3 washes in sterile water. On PDA and OMA plates, three to four pieces of stubble samples were aseptically positioned. The plates then were incubated at 25 ± 1 °C, or room temperature. Any fungus discovered in the inoculum was examined under a microscope to be identified. Any fungus that was recognized as MoT was moved to agar plates for additional culture. To confirm the presence of MoT in wheat stubbles, a molecular approach (PCR technique) was used.

Table 1. DNA concentration of alternative hosts and cereal crops

Sl. No.	Cereals and grass weed species	DNA Concentration (ng/µL)	Absorbance Ratio	
			260/280	260/230
1	Jungle rice	886.4 ± 944.1	1.90	1.89
2	Barnyard grass	1242.4 ± 873.7	1.81	1.51
3	Crab grass	831.2 ± 225.1	1.40	0.83
4	Goose grass	564.7 ± 184.8	2.08	1.97
5	Bermuda grass	228.9 ± 106.2	2.10	1.77
6	Maize	266.3 ± 172.3	1.21	2.26
7	Rice	446.5 ± 512.2	2.01	2.31
8	Wheat (spike)	608.6 ± 324.3	1.76	1.24
9	Wheat stubble	605.4 ± 112.4	1.95	1.49

Note: Negative (uninoculated) control DNA concentrations, where applicable, ranged from 61.7–1502.8 ng/µL with comparable purity ratios to inoculated samples

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Data analysis

Data on disease incidence and severity in the host-range evaluation (Experiment 1) are presented descriptively, as statistical comparison was not applicable given the categorical nature of the result — disease was recorded exclusively in BARI Gom 26, with zero incidence and zero variance across all four replicates in the remaining seven species (see Results). For the stubble survivability data (Experiment 2), spore density values recorded over the first six months of monitoring (prior to complete loss of detectable inoculum) were analyzed by one-way analysis of variance (ANOVA) under a completely randomized design (CRD) with four replications, using R software (version 4.x; R Core Team) [33] with the *agricolae* [6] package for post-hoc mean separation (LSD test, p = 0.05). Treatment means are presented with the standard error (SE) of the mean.

RESULTS

Alternative host evaluation

Blast infection in weeds and cereal crops: Disease incidence and severity for all tested species are summarized in Table 2. Statistical comparison across species was not applicable for this experiment, as disease was observed exclusively in BARI Gom 26, with zero variance recorded among the remaining seven species. None of the five grass weed species, rice, or maize inoculated with MoT spores developed visible blast symptoms, corresponding to 0% disease incidence and 0% severity in all four replicates. In contrast, BARI Gom 26 plants inoculated with the same inoculum developed typical blast symptoms on all ten tagged spikes in every replicate, corresponding to 100% disease incidence. Spikelet-level assessment of the tagged spikes showed a mean disease severity of 84% at 7 days post-inoculation.

Table 2. Disease incidence, severity, and MoT3 PCR detection in wheat, rice, maize, and grass weed species inoculated with MoT, assessed at 7 days post-inoculation

Sl. No.	Plant species	Scientific name	Disease incidence (%)	Disease severity (%)	Typical blast symptoms	MoT3 PCR (361 bp)
1	Wheat (BARI Gom 26)	*Triticum aestivum*	100	84	Yes	+
2	Rice (BRRI dhan28)	*Oryza sativa*	0	0	No	−
3	Maize	*Zea mays*	0	0	No	−
4	Jungle rice	*Echinochloa colona*	0	0	No	−
5	Barnyard grass	*Echinochloa crus-galli*	0	0	No	−
6	Goose grass	*Eleusine indica*	0	0	No	−
7	Crab grass	*Digitaria sanguinalis*	0	0	No	−
8	Bermuda grass	*Cynodon dactylon*	0	0	No	−

Note: +: positive/band detected, −: negative/no band detected in Agarose gel electrophoresis.

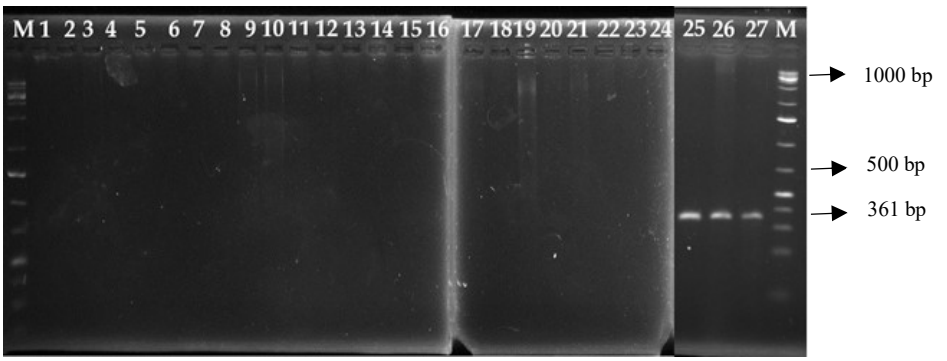

Figure 2. Agarose gel electrophoresis. Lane M: 1 kb ladder left and (far right), Lane 1: Jungle rice (negative control), Lane 2-4: Jungle rice, Lane 5: Barnyard grass (negative control), Lane 6-8: Barnyard grass, Lane 9: Crab grass (negative control), Lane 10-12: Crab grass, Lane 13: Goose grass (negative control), Lane 14-16: Goose grass, Lane 17-19: Bermuda grass, Lane 20-21: Maize, Lane 22-24: Rice, Lane 25-27: Wheat (spike). In the plate, negative control means healthy plants (not inoculated) of the same type

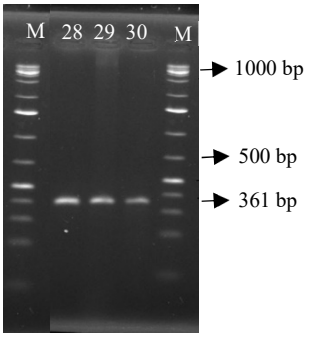

Figure 3. DNA Profile of MoT of three infected wheat stubbles with MoT3 Marker in agarose gel electrophoresis. Lane M: 1 kb

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PCR detection of MoT in weeds and cereal crops: None of the tested grass weed species or cereal crops, except BARI Gom 26, were affected by the wheat blast inoculation at the pre-heading stage, as evidenced by the PCR assay (Table 2). In the PCR detection test, no band was produced for the DNA extracted from any of the inoculated weeds or cereal crops except wheat (Fig. 2). DNA from BARI Gom 26 produced a band of 361 bp size, indicating that BARI Gom 26 was infected when inoculated with MoT. Three bands of equal size were produced for three replications and the bands were monomorphic.

PCR detection of MoT in stubbles: DNA extracted from stubbles of infected wheat when run in PCR with primer, MoT3 yielded a band of 361 bp. The bands were monomorphic and uniform in size (Fig. 3).

Survivability of MoT in wheat Stubbles

Wheat stubbles collected immediately after harvest yielded high MoT spore densities, with a mean of 13.0 × 10^6 ± 0.09 spores/mL suspension recorded in the first month (March) (Fig. 4a,b; Fig. 5). Spore density declined significantly over the following six months ($F_{5,18}$ = 3879.47, $p < 0.001$), from 12.0 × 10^6 ± 0.08 spores/mL in April to 9.75 × 10^6 ± 0.07 in May and 9.25 × 10^6 ± 0.07 in June, followed by a sharper decline to 3.75 × 10^6 ± 0.07 in July and 1.75 × 10^6 ± 0.07 in August ($LSD_{0.05}$ = 0.216, CV = 1.76%). No MoT spores were detected in September, six months after harvest (Fig. 5).

Microscopic examination of stubble samples across the monitoring period showed a corresponding shift in the fungal community present. MoT spores were the dominant fungal structures observed in March and April, with germ tubes and conidial morphology consistent with typical MoT identification (Fig. 6a,b). From June onward, MoT was increasingly observed alongside Alternaria and Fusarium on the same stubble samples (Fig. 6c,d), and by July and August, Alternaria had become visibly dominant in the fungal community as MoT density declined (Fig. 6e). From September onward, only *Fusarium* and *Alternaria* were recovered from stubble samples, with no MoT structures observed (Fig. 6f).

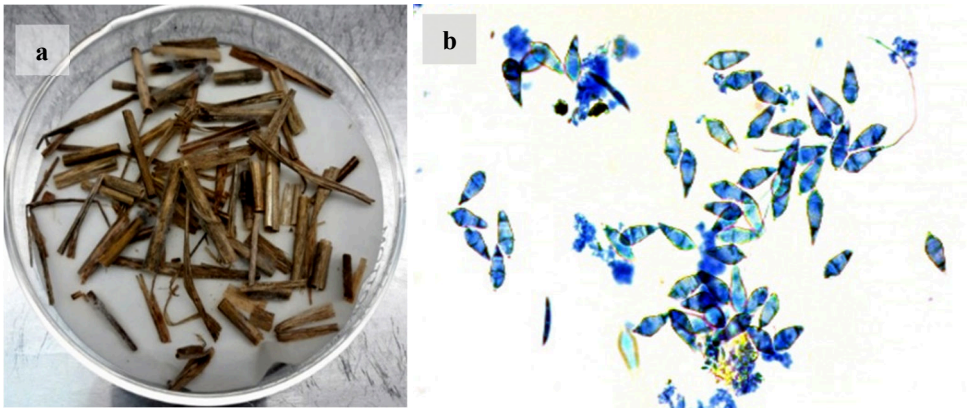

Figure 4. a) Wheat stubbles inocula preparation and incubated in a moist chamber; b) Yielded MoT spores from wheat stubbles just immediately after harvesting of wheat

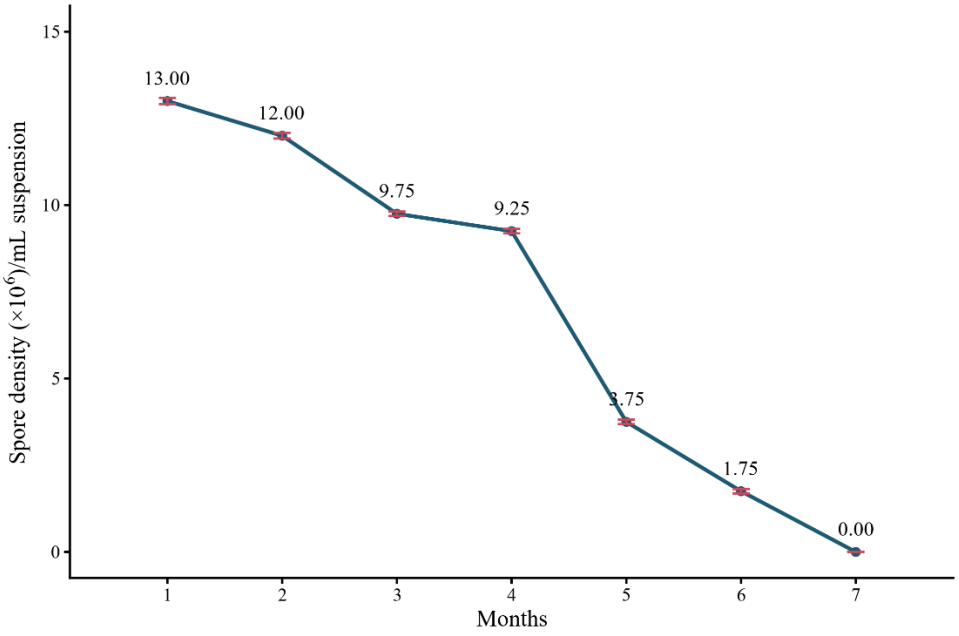

Figure 5. MoT population densities (mean ± SE, n = 4) in wheat stubbles across seven months after harvest. Spore density declined significantly from Month 1 to Month 6 (one-way ANOVA, $F_{5,18}$ = 3879.47, $p < 0.001$; $LSD_{0.05}$ = 0.216), with no spores detected by Month 7.

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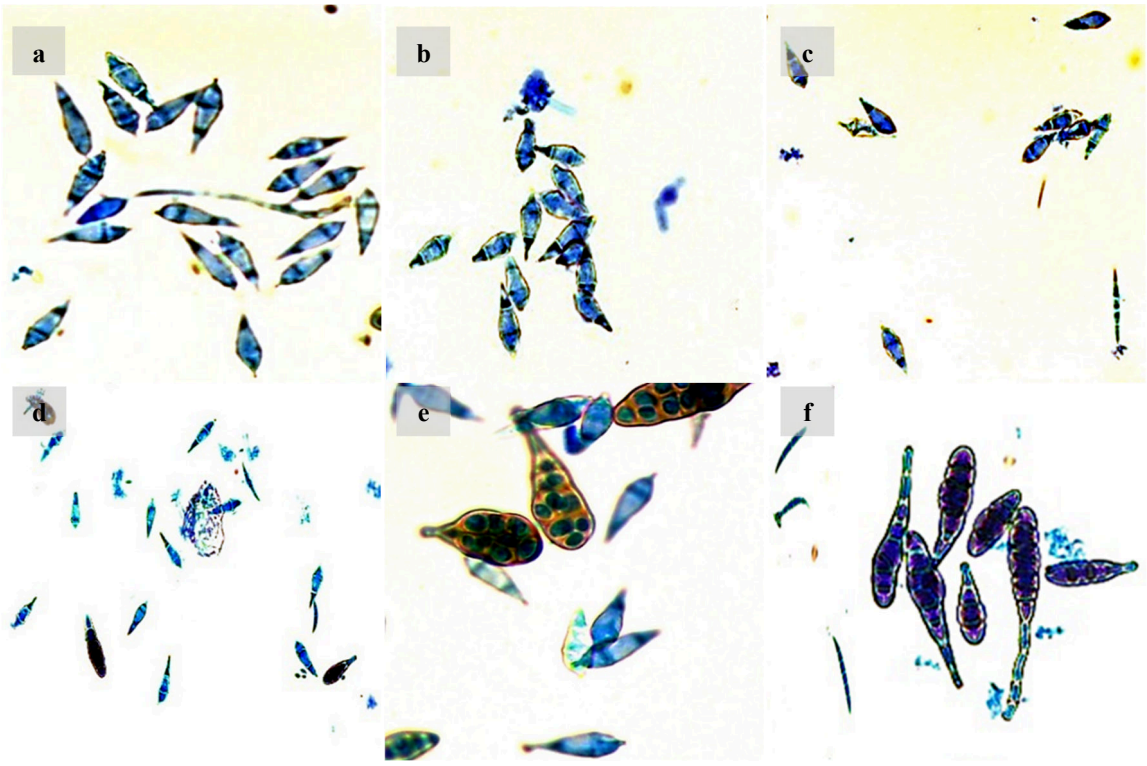

Figure 6. Representative micrographs of MoT spores observed in wheat stubble samples from April to October after harvest, illustrating the visual decline in spore abundance corresponding to the quantitative trend shown in Fig. 5. Spores found in **a**: April (40×), **b**: May (40×), **c**: June (40×), **d**: July (40×), **e**: August (40×), f: September and October (40×), showing predominance of *Alternaria* and *Fusarium* structures with no MoT spores present.

DISCUSSION

Alternative host of wheat blast pathogen: Alternative hosts can significantly influence the epidemiology of plant disease. They can serve as reservoirs for the pathogen, allowing it to persist and multiply even when the primary host (in this case, wheat) is not present. In the present study, none of the five grass weed species, rice, or maize showed disease symptoms or yielded recoverable MoT upon re-isolation following inoculation, suggesting that these species are unlikely to serve as alternative hosts under the experimental conditions used. However, the host range evaluated here was limited to these seven species and does not represent the full diversity of Poaceae occurring in Bangladesh. The selected species were prioritized based on their occurrence in or around wheat-growing areas and their relevance to the cropping systems during the wheat-free period; consequently, the possibility that other grass species or cereal crops may harbour MoT in Bangladesh cannot be excluded.

The absence of infection in these species may reflect host specificity, wherein the genetic makeup of these grasses and cereals lacks the compatibility factors required for MoT recognition and successful colonization. Additionally, constitutive and inducible defense responses in non-host species may actively restrict pathogen establishment, further limiting the host range of the Bangladeshi MoT population.

Previous studies have identified barley (*Hordeum vulgare*), ryegrass (*Lolium* spp.), oats (*Avena sativa*), and other grass species such as wild oats (*Avena fatua*), Italian ryegrass (*Lolium multiflorum*), and Triticale (x *Triticosecale* Wittmack) as potential hosts of wheat blast pathogen. However, the findings of the present study differ from those reported by Farman [7], Hurtado and Toledo [13] in Bolivia and Marangoni *et al.* [25] who reported *Eleusine indica* and *Digitaria* spp. as potential hosts of MoT. These contrasting findings may reflect differences in host susceptibility, pathogen population structure, environmental conditions, and experimental methodology among studies.

Several factors could plausibly account for this discrepancy. First, MoT populations are known to be genetically diverse, and host-specific virulence can vary considerably even within the *Triticum* pathotype [16]; the Bangladeshi MoT population, while originating from a South American lineage [16], may have since diverged in its host range as it adapted to local wheat cultivars, potentially narrowing its ability to infect grass hosts that remained susceptible in the original South American population. Second, the *Eleusine indica* and *Digitaria* spp. populations tested in our study represent local Bangladeshi ecotypes, which may differ genetically from the South American populations of the same species used in prior studies, potentially altering host-pathogen compatibility at the ecotype level. Third, methodological differences between studies —

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including inoculum concentration, isolate origin, plant growth stage at inoculation, and environmental conditions during the infection window — can each independently influence infection outcomes in host-range trials, making direct comparison across studies conducted in different countries and pathogen populations inherently uncertain. Given these considerations, our findings should be interpreted as evidence that these species are not universal hosts of MoT, rather than as a contradiction of the South American reports, which remain valid for the populations and conditions under which they were generated.

Although barley, a close relative of wheat, has been identified as a potential alternative host for MoT [36], and studies have shown that the pathogen can infect and cause disease symptoms in barley plants, this finding is of limited local concern because barley is cultivated only on a very small scale in Bangladesh and generally outside the principal wheat-growing regions, reducing its likely epidemiological importance under local conditions. Similarly, ryegrass has been identified as a potential alternative host [12], but it is not commonly cultivated or naturally established in Bangladesh, further limiting its relevance to local disease dynamics.

Survivability of blast in wheat stubbles: Wheat stubble is an important source of inoculum between successive wheat-growing seasons. It comprises residual plant material remaining after harvest, including stems, leaves, and other plant debris, on which MoT can survive during the wheat-free period. In our study, stubble left in the field consistently produced detectable MoT spores for five to six months, with densities gradually declining thereafter until reaching undetectable levels.

Several factors may explain the observed decline in MoT spore abundance over time. As wheat stubble decomposes, it becomes a progressively less suitable substrate for pathogen survival, while increasing competition from saprophytic microorganisms may further reduce MoT persistence. It should be noted that the relative abundance of *Alternaria* and *Fusarium* on stubble samples was assessed descriptively based on microscopic observation, rather than through quantified colony counts or isolation frequency; future studies incorporating quantitative measures of competing saprophytic fungi would help clarify their role in MoT decline on stubble.

The progressive replacement of MoT by *Alternaria* and *Fusarium* on decomposing wheat stubble observed in this study likely reflects a broader ecological succession process common to saprophytic colonization of crop residues. As stubble ages and its nutrient composition changes, faster-growing or more competitive saprophytic fungi such as *Alternaria* and *Fusarium* spp. are known to outcompete more specialized pathogens for the remaining substrate, through mechanisms that may include resource competition, production of antifungal metabolites, or direct mycoparasitism [25, 35]. This successional replacement may partly explain the observed decline in MoT spore density independent of substrate depletion alone, suggesting that the stubble microenvironment becomes progressively less favorable for MoT survival as competing saprophytes establish dominance. This raises the possibility that naturally occurring or introduced antagonistic fungi could be explored as a component of integrated stubble management, though this would require targeted investigation beyond the scope of the present study.

The findings are in agreement with Castroagudin *et al.* [5] who opined that *Pyricularia graminis-tritici* (blast fungus) survived and generated perithecia, or sexual fruiting bodies, on crop residues in between wheat crop seasons. Additionally, according to Sadat and Choi [35], local officials received recommendations for the removal of crop residues from wheat, barley, millet, and oats with the subsequent introduction of wheat blast into South-East Asia. The current results, however, differ from those of Maciel *et al.* [22], who noted the blast fungus's sporulation on wheat residues for up to five months and came to the conclusion that there was very little chance the causal agent of wheat blast could survive from one crop to another in wheat residues in Brazilian conditions.

Based on our findings, we propose the following specific management recommendations for wheat blast control in Bangladesh. First, since none of the tested grass weeds or cereal crops served as alternative hosts, our findings suggest that infected wheat stubble may represent a more important source of MoT survival than the five grass weed species and two cereal crops evaluated in this study. Given that MoT spores remained detectable in stubble for up to six months post-harvest, management practices aimed at reducing inoculum in infected wheat residues, including timely removal or appropriate residue management after harvest, may help reduce primary inoculum before the subsequent wheat-growing season. Second, the roughly seven-month wheat-free period in the standard Bangladeshi cropping sequence (wheat–jute/*aus* rice or maize–*aman* rice) provides an opportunity for residue management practices that may reduce pathogen survival before the following wheat season. Third, continued deployment and development of blast-resistant or -tolerant wheat cultivars, alongside cultivar mixtures shown to maintain yield under high disease pressure [20], remains an essential complementary strategy, particularly given the limited efficacy of fungicides once spike infection occurs. These residue-management practices should be integrated with the deployment of blast-resistant cultivars, timely fungicide application, and weather-based early warning systems [18] to strengthen integrated wheat blast management in Bangladesh.

However, because only five grass weed species together with rice and maize were evaluated in this study, further multi-year investigations involving a broader range of Poaceae species and agroecological regions are required before the relative importance of different inoculum sources can be fully established.

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This study provides new evidence that the five grass weed species, rice, and maize evaluated are unlikely to serve as alternative hosts of MoT under the experimental conditions used, whereas infected wheat stubble remained a potential source of inoculum for up to six months after harvest. Although additional multi-year studies involving a broader range of Poaceae species and agroecological regions are required, these findings improve our understanding of MoT survival in Bangladesh and support timely residue management as an important component of integrated wheat blast management.

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