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Efficacy of Ethanol Extract of Botanicals in Controlling Wheat Blast Fungus *Magnaporthe oryzae triticum in vitro*

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Abstract

The present research work was conducted to evaluate the efficacy of eight fresh botanicals namely *Azadirachta indica* (Neem leaf), *Allium cepa* (Onion bulb), *Allium sativum* (Garlic), *Curcuma longa* (Turmeric), *Zingiber officinale* (Ginger), *Allamanda cathartica* (Allamanda leaf), *Nigella sativa* (Black cumin) and *Aloe vera* (Aloe vera) against *Magnaporthe oryzae triticum* (MoT) and solvent, ethanol (95%) was used for the phytochemical extraction of various plant parts. Three concentrations namely 1:1 (w/v), 1:0.50 (w/v) and 1:0.25 (w/v) of plant parts and ethanol were used for botanical extraction. The antifungal activity of botanicals against a virulent MoT isolate CHMoT07 was evaluated *in-vitro* using poison food technique. The lowest mycelia growth was recorded with *Aloe vera* (Aloe vera leaf) extracts and *Nigella sativa* (Black cumin seeds) extracts @ 1:1 w/v and @ 1:0.25 w/v with growth rate of 3.00 mm and 3.33 mm respectively at 7 days after inoculation, whereas the highest mycelia growth rate of MoT isolates was recorded in control plates both at 7 DAI and 14 DAI under *in-vitro* condition.

Keywords: Wheat Blast, *Magnaporthe oryzae triticum,* botanical extracts, *in vitro*

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Introduction

Wheat (*Triticum aestivum*) is recognized as one of the most important cereal crops within the world [1]. On the national economy, it is typically a human food grain and created optimistic impact globally. Wiese (1987) [2] mentioned that, wheat provides about 20% of the world food calories and nearly 40% of the total world population consumed it as staple food. Origin of wheat is from the Levant region of the Near East however currently cultivated worldwide. Thus, being larger than any other crop, wheat is grown on more than 701.5 million hectares [3]. In 2017, wheat ranked as the third most-produced cereal in the world with the production of 771.7 million tons [4] through China (131.4 million tons), India (99.7 million tons), Russia (72.1million tons) and USA (51.3 million tons) were the four largest wheat producers in 2018 [5].

According to the Department of Agricultural Extension, Bangladesh in 2016, total wheat cultivated area in Bangladesh is about 498,000 ha. In the year of 2016 an outbreak of wheat blast was reported for the first time in Bangladesh particularly in the districts of Kushtia, Meherpur, Chuadanga, Jhenaidah, Jessore, Barisal, Bhola, Magura, Narail, and Faridpur [6]. It was estimated that 15% area were affected around 101,660 ha of cultivated wheat area by a devastating wheat blast in the same year. The incidence and severity of wheat blast associated with yield losses among completely different districts varied considerably. The prevalence of wheat blast was higher in Meherpur (70%) although the very best average yield loss (51%) was recorded in Jhenaidah. The average yield loss was lower than 51% across districts and in several cases,100% yield losses were recorded in individual wheat fields [7].

The area beneath wheat cultivation currently extends to concerning 1.78 lakh ha and the annual production is about 10 lakh metric tons [8]. Some severely infected fields were burned due to approximately 15% of Bangladesh's total wheat-affected area, which reduced 15% wheat production in nine infected districts [6, 7, 9]. In spite of such a decrease, in 2016 compared to that of 2015, 2.7% of total wheat production in Bangladeshis increased (35,000 metric ton [MT]). A rise in total harvested areas (420,000-425,000 ha) and increasing yields (3.10-3.14 MT/ha) contributed to the overall wheat production in 2016 [10].

Use of fungicides against wheat blast wasn't therefore effective and chemical application conjointly caused environmental pollution and toxicity to beneficial soil microbes, higher plants furthermore animals and human

beings. It has also enhanced production costs of farmers [11] and natural biological systems have disrupted over decades because of their continual use and sometimes resulted in the development of fungal resistance besides producing undesirable effects on non-target organisms, and also fostered environmental and human health considerations [12]. Rout and Tewari (2012) [13] stated in their study the potentiality of integrating in the management of economically important diseases, the products prepared from green plants should be preferred as they are environmentally safe, non-pollutive, non-toxic and non-hazardous to beneficial microorganisms. Allamanda (*Allamanda cathertica*) leaves are the promising source of many antifungal compounds with medicinal properties [14]. The antifungal effect of the extracts of Neem (*Azadirachta indica* L.), Garlic (*Allium sativum* L.) and Calatropis (*Calotropis procera* L.) against *Magnapothe oryzae triticum* (MoT) was evaluated by Khanzada and Shah [15] following poisoned food technique. They found complete growth inhibition of the test fungus in a higher dose of garlic extract in *in vitro* bioassay. Development of fungicidal resistance into the pathogens and residual toxicity in soil and in the crop plants were owing to frequent use of fungicides. On the other hand, some botanical pesticides and bio-control agents have tested to be reliable and don't have any adverse effect on environment [16, 17]. Mondol *et al*. 2018 [18] studied that chitosan (0.4%), salicylic acid (9mM) and benzoic acid (9mM) are effective in suppressing the growth of MoT. Chitosan, salicylic acid and benzoic acid are bio-polymers and not harmful for ecosystem and are completely safe for human health. Therefore, these bio-polymers can be utilized as alternative to chemical fungicides for wheat blast management.

Therefore, to control this devastating pathogen MoT ecofriendly management with non hazardous botanical extracts are going to be most effective until crop improvement with desired resistance gene against this pathogen in Bangladesh. The present research work was thus undertaken to find out the antifungal effect of some botanical extracts against MoT *in vitro*.

Materials and Methods

The present investigations were carried out under laboratory conditions from January to July, 2019 to ascertain the incidence, severity of wheat blast and *in-vitro* evaluation of botanicals against MoT in the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka.

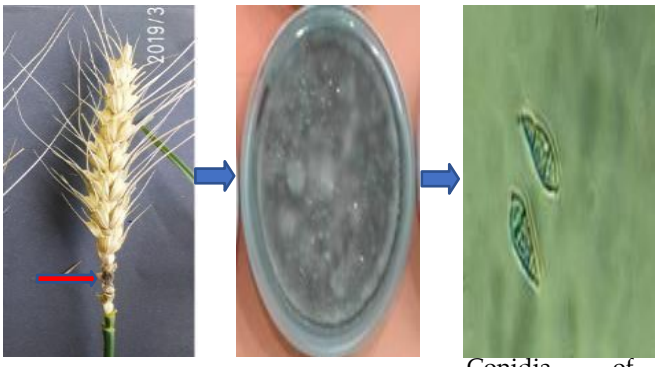

Figure 1. Flow chart of isolation, identification, and culture of *Magnaporthe oryzae triticum* on OMA media

Diseased plant samples were collected from infected wheat fields and preserved at 4° C temperature in the laboratory of SAU for isolation. Standard blotter method [19] was followed for the isolation of MoT pathogen from infected wheat spikes. The water agar was prepared by mixing 20 g agar with 1000 ml distilled water, and potato dextrose agar media was made consisting of 200 g peeled potatoes, 20 g dextrose, and 20 g agar combined with 1000 ml distilled water were used for the isolation of blast pathogen. Appropriate size (15-20 cm in size) of diseased spikes infected with pathogen of wheat cultivars were cut around the area showing the blast lesion and were surface sterilized with 1% sodium hypochlorite (NaOCl) for 1 minute followed by 3 times washes with sterile distilled water. To encourage sporulation, the plant pieces were placed in petri dishes lined with moist filter papers and incubated at 26±1°C for 24 hours. After incubation, these infected spike pieces were examined under stereo-dissecting microscope (Motic, China). Abundant sporulation with grey, dense and bushy appearance was observed in and around the lesions. Single conidium was picked out using a sterile moistened needle across the sporulating lesion observing under the stereo microscope. The conidia were placed on water agar for further growth experimentation. After 12 hours, mycelium was visible in petri dish under the stereo microscope and then the hyphal tip was cut and placed in potato dextrose agar media plates containing Streptomycin (40 mg/L) and pure culture of MoT were prepared by incubating there in 26±1°C. The marginal mycelial growth that developed subsequently was picked-up aseptically for sub-culturing until a pure culture of MoT was obtained. The culture was kept under 12 hrs light and 12 hrs darkness conditions for sporulation. MoT isolate was identified by three-celled, pyriform, light-colored conidia **(Figure 1)**.

Figure 2. Botanicals used in controlling mycelial growth of *Magnaporthe oryzae triticum* in-vitro. Extracts of A. *Allium cepa*, B. *Allium sativum*, C. *Curcuma longa*, D. *Zingiber officinale*, E. *Azadirachta indica*, F. *Nigella sativa*, G. *Allamanda cathartica*, H. *Aloe vera*

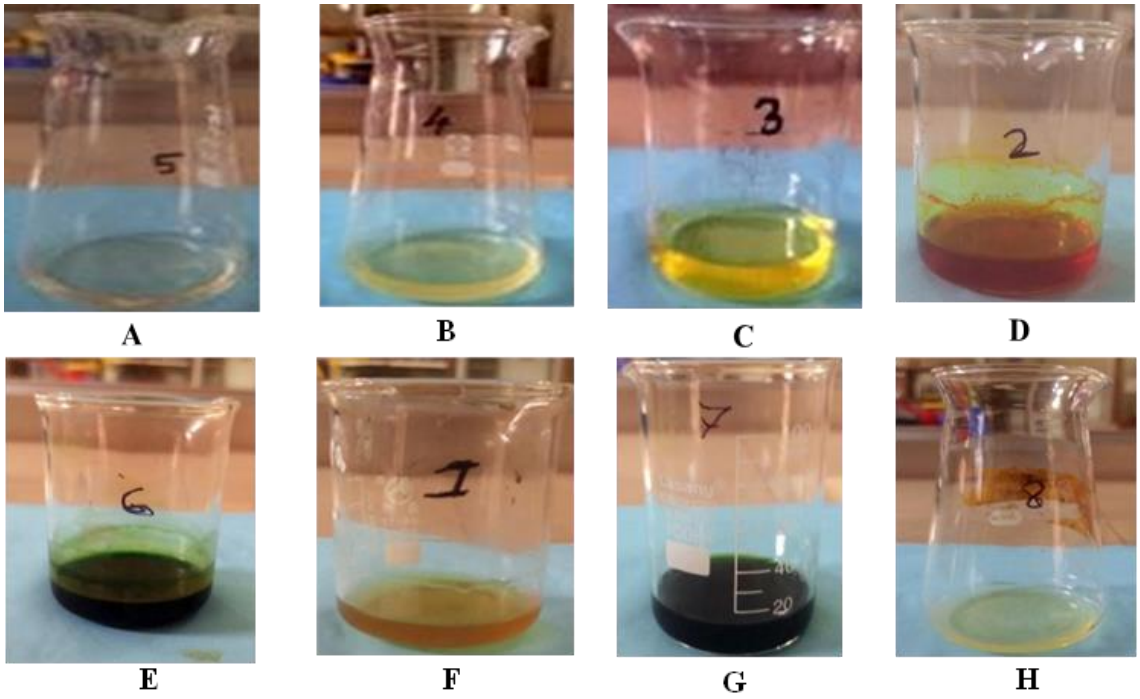

Figure 3. Botanical extracts used in controlling mycelial growth of *Magnaporthe oryzae triticum in-vitro.* Extracts of A. *Allium cepa,* B. *Allium sativum,* C. *Curcuma longa,* D. *Zingiber officinale,* E. *Azadirachta indica,* F. *Nigella sativa,* G. *Allamanda cathartica,* H. *Aloe vera*

The pure culture was maintained by subculturing at an interval every 15 days and preserved at low temperature (4ºC) in refrigerator.

Fresh plant parts namely *Azadirachta indica* (Neem leaf), *Allium cepa* (Onion bulb), *Allium sativum* (Garlic), *Curcuma longa* (Turmeric), *Zingiber officinale* (Ginger), *Allamanda cathartica* (Allamanda leaf), *Nigella sativa* (Black cumin) and *Aloe vera* (Aloe vera) were used as treatments **(Table 1 & Figure 2)**. 95% ethanol solvent was used for the phytochemical extraction of various plant parts [20]. Three concentration 1:1 (w/v), 1:0.50 (w/v) and 1:0.25 (w/v) of ethanol was used for botanical extraction. For 1:1 (w/v) concentration extraction with ethanol, 100g of plant materials was dissolved in 100 ml ethanol. To avoid evaporation and subjected to filtration through sterilized Whatman no.1 filter paper the mixture was kept undisturbed at room temperature in a sterile flask covered with aluminum foil for 24 hrs.

Table 1. Botanicals used in controlling mycelia growth of *Magnaporthe oryzae triticum in-vitro*

Name of botanicals			Concentration used
Scientific name	English name	Plant parts used	
Allium cepa	Onion	Bulb	1:0.25(w/v) 1:0.50 (w/v) 1:1 (w/v)
Allium sativum	Garlic	Clove	1:0.25(w/v) 1:0.50 (w/v) 1:1 (w/v)
Curcuma longa	Turmeric	Rhizome	1:0.25(w/v) 1:0.50 (w/v) 1:1 (w/v)
Zingiber officinale	Ginger	Rhizome	1:0.25(w/v) 1:0.50 (w/v) 1:1 (w/v)
Azadirachta indica	Neem	Leaf	1:0.25(w/v) 1:0.50 (w/v) 1:1 (w/v)
Nigella sativa	Black cumin	Seed	1:0.25(w/v) 1:0.50 (w/v) 1:1 (w/v)
Allamanda cathartica	Allamanda	Leaf	1:0.25(w/v) 1:0.50 (w/v) 1:1 (w/v)
Aloe vera	Aloe vera	Leaf	1:0.25(w/v) 1:0.50 (w/v) 1:1 (w/v)
Control	No botanical extracts		

After filtration, the extract was evaporated in water bath until 100 ml extract was left in the container **(Figure 3)**. For 1:0.50 (w/v) and 1:0.25 (w/v) 100 g of plant materials were dissolved in 50 ml and 25 ml ethanol, respectively [20]. Ethanolic extract thus obtained were immediately evaluated for antifungal activities using the poisoned food technique [21].

PDA plates were amended with different concentration (1:1 w/v, 1:0.50 w/v, 1:0.25 w/v) of botanical extracts separately. The plates were inoculated with 5 mm fungal blocks with the help of sterilized needle, MoT and these blocks were transferred to the center of the petri plates. The mycelial growth of MoT was recorded at seven days after inoculation by measuring the average of two diameters at right angles (90 degrees) to one another. Three replications were maintained for each treatment and the mean radial mycelial growth was considered for measuring each treatment. The effect of plant extract was calculated as percent growth inhibition using the following formula as adopted by [22, 23].

% inhibition = (dc – dt)/dc × 100

Where dc = Average increase in mycelial growth in control, dt = Average increase in mycelial growth in treatment.

Statistical analysis

A completely randomized design (CRD) with three replications was applied in this experiment. Analysis of data of different parameters was subjected to perform by statistical analysis using R software version 3.6.0 [24].

Results

All botanicals performed significantly effective against MoT isolate in lessening mycelial growth compare to control **(Table 2)**. The result revealed that *Aloe vera* (Aloe vera leaf) extracts was found most effective in reducing the mycelial growth at 7 days but in 14 days *Nigella sativa* (Black cumin seed) extracts and *Aloe vera* (Aloe vera leaf) extracts both botanicals significantly reduced the mycelial growth of MoT.

Table 2. Efficacy of botanicals on mycelial growth of *M. oryzae triticum*

Treatments	Radial mycelial growth (mm)		% Growth reduced over control	
	7 DAI	14 DAI	7 DAI	14 DAI
Allium cepa	35.22b	42.00d	28.93	38.93
Allium sativum	21.67e	52.56b	56.27	23.58
Curcuma longa	26.78d	47.11c	45.94	31.50
Zingiber officinale	26.78d	36.78f	49.96	46.52
Azadirachta indica	31.56c	52.00b	36.31	24.39
Nigella sativa	4.11g	10.00g	91.70	85.46
Allamanda cathartica	26.22d	40.33e	47.09	41.36
Aloe vera	5.22f	8.89g	89.46	87.07
Control	49.56a	68.78a		
CV (%)	3.68	3.02		

In a column treatment means with the same letter are not significantly different. DAI= Days after inoculation

Table 3. Effect of different concentration levels of botanicals on mycelial growth of M. oryzae triticum

Concentration of botanicals	**Radial mycelial growth (mm)**	
	7 DAI	**14 DAI**
1:0.25 w/v @0.1%	30.37a	47.59a
1:0.50 w/v @0.2%	25.89b	40.74b
1:1 w/v @0.4%	19.44c	31.15c
CV (%)	3.68	3.02

In a column treatment means with the same letter are not significantly different.
DAI=Days after inoculation

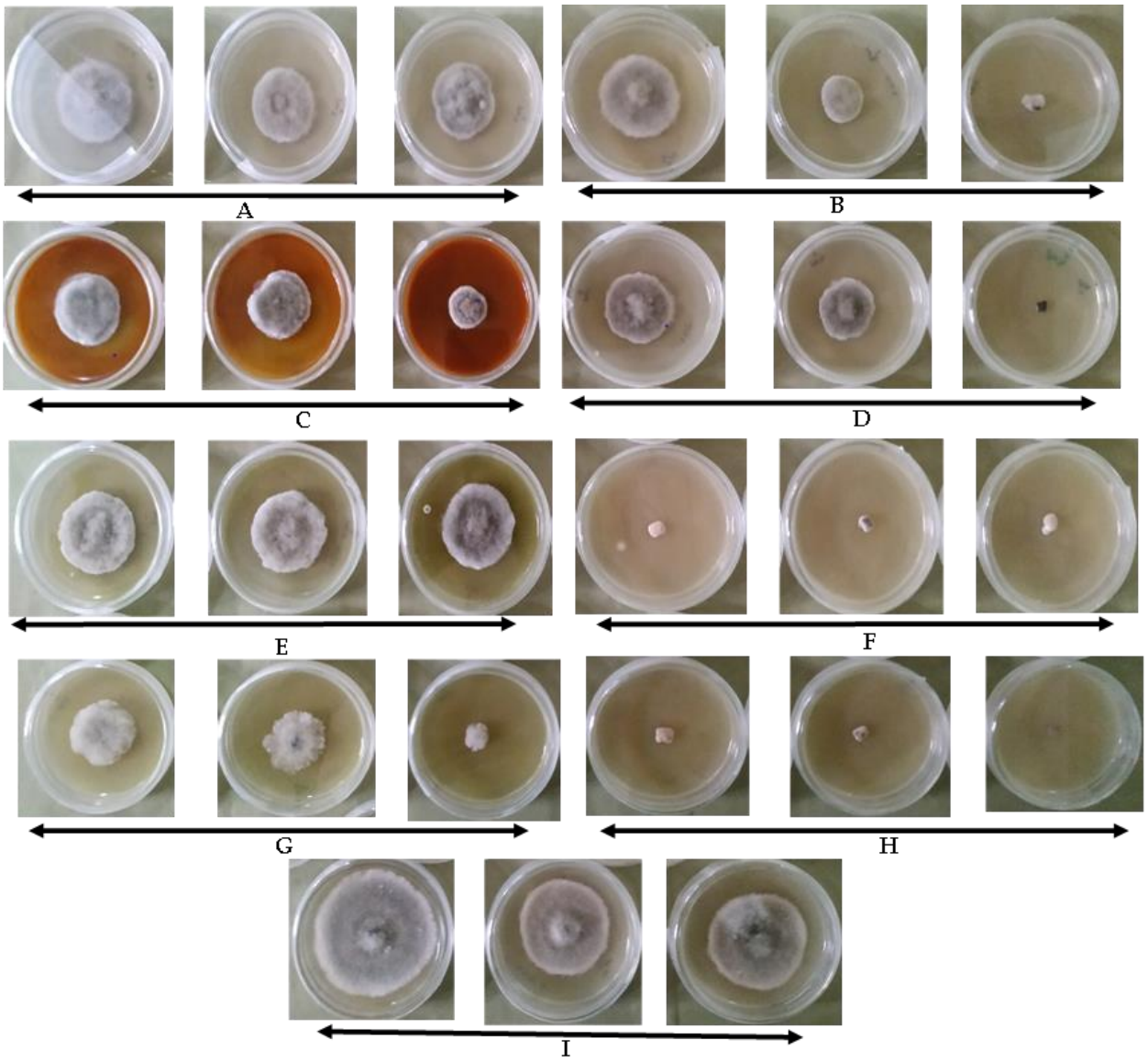

Figure 4. Mycelial growth, color and appearance of *M. oryzae triticum* on PDA media supplemented with ethanol extracts of different botanicals (7 DAI). A. *Allium cepa,* B. *Allium sativum,* C. *Curcuma longa,* D. *Zingiber officinale,* E. *Azadirachta indica,* F. *Nigella sativa,* G. *Allamanda cathartica,* H. *Aloe vera,* I. Control

Three concentration level of botanicals (1:0.25 w/v @ 0.1%, 1:0.50 w/v @ 0.2%, 1:1 w/v @ 0.4%) was tested against the pathogen and (1:1) found the best concentration in both 7 DAI and 14 DAI **(Table 3)**. With the concentration increase of all tested plant extracts, the mycelial growth of MoT decreases.

The growth characteristics like media color, colony color and shape of colony of MoT on PDA media were noticed supplemented with different plant extracts. In case of PDA media, the colony color of MoT was grey ash centre and black margin. In contrast, PDA media supplemented with *Azadirachta indica* (Neem leaf) extract was showed white with grey, *Allamanda cathartica* (Allamanda leaf) extract, *Aloe vera* (Aloe vera) leaf extract, and *Nigella sativa* (Black cumin) seed extracts showed white colony color. *Allium cepa* (Onion bulb) extracts, *Allium sativum*(Garlic) extracts and *Curcuma longa* (Turmeric rhizome) extracts and *Zingiber officinale* (Ginger) showed rhizome extract showed grey with white color colony. This result showed that PDA media supplemented with totally different plant extracts have an effect on the colony color of *M. oryzae triticum* **(Figure 4 and Table 4).**

Antimicrobial activities of eight botanicals with specific concentration were assayed and results on presented in **Table 4**. The data revealed that botanicals were found significant in suppression of mycelia growth at higher concentration over untreated check in the fungal pathogens.

Antimicrobial activities of eight botanicals with specific concentration were assayed and results on presented in **Table 4**. The result reveals that the minimum radial mycelial growth was recorded from *Nigella sativa*: 1:1

Table 4. Effects of ethanol extracts of botanicals on mycelia growth and colony characters of *Magnaporthe oryzae triticum*

Treatments	Ethanol botanicals ratio (w/v)	Radial mycelia growth (mm)		Colony character	
		7 DAI	14 DAI	Colony color	Shape
Allium cepa	1:0.25	38.33b	58.33c	Gray ash	Regular
	1:0.50	36.00c	41.67hi		
	1:1	31.33f	26.00l		
Allium sativum	1:0.25	31.00f	60.33bc	Gray ash	Regular
	1:0.50	23.00i	56.33d		
	1:1	11.00m	41.00i		
Curcuma longa	1:0.25	33.33e	59.00bc	Gray with white margin	Regular
	1:0.50	29.00g	49.00f		
	1:1	18.00k	33.33j		
Zingiber officinale	1:0.25	34.00de	46.33g	Gray with white margin	Regular
	1:0.50	26.67h	40.33i		
	1:1	19.67j	23.67m		
Azadirachta indica	1:0.25	39.00b	61.00b	Grey with white	Regular
	1:0.50	30.33fg	51.67e		
	1:1	25.33h	43.33h		
Nigella sativa	1:0.25	5.00o	11.33n	White	Regular
	1:0.50	4.33opq	9.67no		
	1:1	3.00q	9.00o		
Allamanda cathartica	1:0.25	35.00cd	51.00e	White	Regular
	1:0.50	30.00fg	40.33i		
	1:1	13.67l	29.67k		
Aloe vera	1:0.25	7.67n	11.67n	White	Regular
	1:0.50	4.67op	9.00o		
	1:1	3.33pq	6.00p		
Control	Only PDA	50.00a	69.33a	Grey with white	Regular
	Only PDA	49.00a	68.67a		
	Only PDA	49.67a	68.33a		
CV%		3.68	3.02		

In a column treatment means with the same letter are not significantly different DAI= Days after inoculation

w/v @ 0.4% (3.00 mm) combination at 7 days culture age which is statistically similar with *Aloe vera*: 1:1 w/v @ 0.4% (3.33mm) and *Nigella sativa*: 1:0.50 w/v @ 0.2% (4.33 mm) combination. Control plates (only PDA: 1:0.50 w/v @ 0.2% and only PDA: 1:1 w/v @ 0.4%) were always observed the highest growth of mycelium. At 14 days culture age, lowest mycelial growth was observed in *Aloe vera*: 1:1 w/v @0.4% combination (6.00 mm). In 2nd lower growth recorder from *Aloe vera*: 1:0.50 w/v @0.2% (9.00 mm) and *Nigella sativa*: 1:1 w/v @0.4% (9.00) combination, which were statistically identical to each other. Control plates (only water amended PDA: (50.00), (49.00) and (49.67)) were observed highest growth of mycelium both at 7 days culture age and 14 days culture age. The highest concentration of botanical extracts was more effective compared to low concentration in reducing the radial mycelia growth of the fungus as observed in case of blast pathogen. With the concentration increase of all tested botanical extracts, the mycelial growth of MoT reduced compared to control.

Discussion

In Bangladesh about 80 % of people lean on directly on agriculture for their food and livelihood, with wheat being the second most important crop after rice. Wheat blast is caused by MoT is a new disease in Bangladesh which may cause up to 100% yield loss of wheat. In another study significant losses in grain yield due to wheat blast were observed in all the survey sites of the two south-western districts of Bangladesh (Tanjina et al., 2019) [25].

The result evaluated that the lowest radial mycelial growth was recorded from *Nigella sativa*: 1:1 w/v @ 0.4% (3.00 mm) combination at 7 days culture age which is statistically similar with *Aloe vera*: 1:1 w/v @ 0.4% (3.33mm) and *Nigella sativa*: 1:0.25 w/v @ 0.2% (4.33 mm) combination. Control plates (only PDA) were always observed highest growth of mycelium. At 14 days culture age, lowest mycelial growth was observed in Aloe vera: 1:1 w/v @ 0.4% combination (6.00 mm). In 2nd lower growth recorder from Aloe vera: 1:0.25 w/v @0.2% (9.00 mm) and *Nigella sativa*: 1:1 w/v @0.4% (9.00) combination, which were statistically identical each other. Control plates (only PDA (50.00), (49.00) and (49.67)) were observed highest growth of mycelium both at 7 days culture age and 14 days culture age. The findings are similar to Zohura et al. (2018) [26] who reported the effectiveness of 12 plant extracts namely neem leaf extract, bishkatali leaf extract, nishinda leaf extract, allamonda leaf extract, acasia leaf extract, tulsi leaf extract, mehendi leaf extract, datura leaf extract, bishkochu leaf, black cumin seed extract, garlic clove extract and mehogoni seed extracts against MoT *in vitro*. Among these twelve plant extracts, four plant extracts viz. Tulsi leaf extract, Mehendi leaf extract, Datura leaf extract and Garlic clove extract impede the highest percentage (93.75%) of mycelial growth followed by Black cumin seed extracts (90%) at 10 DAI where lowest percentage of mycelial growth inhibition (7.5%) were recorded in Allamonda leaf extract over control.

The highest concentration of botanicals extracts was more effective compare to low concentration in reducing the radial growth of the fungus as observed in case of blast pathogen. The results are similar with the findings of

Zohura *et al.,* (2018) [26] where no growth inhibition had taken placed on control plate. Allamonda leaf extract performed with the minimum percent growth inhibition (7.5%) compared to control while the maximum inhibition was observed in the plates containing Garlic clove, Tulsi leaf, Mehendi leaf (93.75%) and Black cumin seed extract (90%) respectively after 10 days of inoculation. This result is also in conformity with the findings where they stated that *H. anthelminthicus* fruit extracts exhibited antifungal potential to growth inhibition at the 10,000-ppm concentration and 100% growth inhibition against *Pyricularia oryzae*, *P. palmivora* and *R. solani* were noted followed by *S. rolfsii* at 96.33% when compared with water control [27].

For controlling the rice blast disease In-vitro and in-vivo experiment were carried out by Hubert et al. (2015) [28] where the effects of *Aloe vera, Allium sativum, Annonamuricata, Azadirachta indica, Bidenspilosa, Camellia sinensis, Chrysanthemum coccineum*, processed *Coffee arabica, Datura stramonium, Nicotiana tabacum* and *Zingiber officinalis* extracts for control of rice blast disease (*Magnaporthe oryzae*) both were assessed. At 10% and 25% (v/v) the highest (81.12%) and (89.40%) inhibitory effect was observed in processed *C. arabica* against *P. grisea*, respectively. To manage rice blast disease these plant extracts can be used.

Conclusions

Wheat blast caused by *Magnaporthe oryzae triticum* (MoT) recognized as a devastating disease and caused up to 100% yield loss first time in some wheat growing areas of Bangladesh in the year 2016. As there are no appropriate control measures have been developed till now so the effective management of this pathogen is the environment friendly management with botanical extracts until the establishment of resistance cultivars against this notorious pathogen in Bangladesh.

The highest radial mycelial growth inhibition *Aloe vera* (Aloe vera leaf) extracts and *Nigella sativa* (Black cumin seeds) extracts 1:1 w/v @ 0.4% (3.00mm and 3.33mm) concentration at 7 days after inoculation, whereas the lowest inhibition *Allium cepa* (onion) extracts 1:0.25 w/v @ 0.1% (38.33mm) concentration of MoT under *in-vitro* condition. However, this experiment with plant extracts urgent to be invented out to assess the field efficacy of these botanical extracts with different concentrations and frequencies in controlling blast of wheat.

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Conflict of interest

The authors declare no conflict of interest.

Ethical issues

There are no ethical issues involved in this research work.

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Author contributions

M. K. Rehena collected wheat blast samples from the field, conducted the research and wrote the article; F. M. Aminuzzaman designed and supervised the study and edited the manuscript; M. L. Ashrafi and U. A. Habiba collected the blast samples from field and analyze the data; M. S. M. Chowdhury, Z. Nazifa and M. Ahmed read the manuscript contributed to the conceptualization, and methodology of the study.

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