**Fundamental and Applied Agriculture**
Vol. 3(2), pp. 422–433: 2018
doi: 10.5455/faa.293640

*Plant Pathology*
ORIGINAL ARTICLE

# Effects of plant extracts on controlling wheat blast disease caused by *Magnaporthe oryzae* Pathotype *triticum* in Bangladesh

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ARTICLE INFORMATION

*Article History*
Submitted: 13 March 2018
Revised: 29 March 2018
Accepted: 01 April 2018
First online: 04 April 2018

*Academic Editor*
Md Mamunur Rashid

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ABSTRACT

The present research work was conducted to evaluate the efficacy of twelve plant extracts viz. Neem (*Azadirachta indica*), Bishkatali (*Polygonum hydropiper*), Nishinda (*Vitex negundu*), Allamonda (*Allamanda cathertica*), Acasia (*Acacia auriculiformis*), Tulsi (*Ocientific tenuiflorum*), Mehendi (*Lmetawsonia alba*), Datura (*Datura metel*), Bishkochu (*Alocasia fornicate*), Black cumin (*Nigella sativa*), Garlic (*Allium sativum*), Mehogoni (*Swietenia macrophylla*) @ 1:10 along with two fungicides Provax (Provaxaltonin) and Nativo (Trifloxystrobin + Tebuconazole) @ 0.2% as check against *Magnaporthe oryzae* Pathotype *triticum* (MoT) which is responsible for wheat blast disease at Department of Plant Pathology, Bangladesh Agricultural University and Plant Pathology Division, Bangladesh Institute of Nuclear Agriculture. In the laboratory experiment, the efficacies of plant extracts were evaluated by the measurement of percent inhibition of radial mycelial growth of MoT. The highest percentage of mycelial inhibition (93.75%) was recorded in case of four plant extracts namely Tulsi, Mehendi, Datura and Garlic followed by Black cumin seed extracts (90%) at 10 days after inoculation, where as Allamonda leaf extract showed lowest percentage of mycelial growth inhibition (7.5%). In *in vitro* test, minimum percentage of disease incidence and severity were recorded in case of Garlic clove extract (16.28% and 3.5%) treated plants and the Mehendi leaf extract treated plants showed highest percent of disease incidence and severity (66.0% and 68.0%). Garlic clove extracts also showed best performance for yield contributing parameters namely ear length (9.20 cm), number of ear/pot (13.25), number of healthy ear/pot (13.0), number of total and healthy spikelets/ear (34.20 and 33.40), number of total and healthy grains/ear (23.00 and 21.80) and weight of 1000 total and healthy grains/pot (56 and 52 g) followed by Black cumin, whereas Mehendi leaf extract treated plants showed lowest value for all the yield contributing parameters. Both in in vito and pot experiment Garlic extract showed best performance and it might be used for the eco-friendly management of blast disease and increase the yield of wheat.

**Keywords:** Wheat blast, *Magnapothe ozyzae*, Plant extract, Efficacy, Bangladesh

*Cite this article*: Zohura FT, Kashem MA, Hasan MAI, Hossain MA. 2018. Effects of plant extracts on controlling wheat blast disease caused by *Magnaporthe oryzae* Pathotype *triticum* in Bangladesh. Fundam Appl Agric 3(2): 422–433. doi: 10.5455/faa.293640

## 1 Introduction

Bangladesh is an agricultural country where different types of agricultural crops are grown. Cereal crops are main cultivated crops here. After rice, wheat is the second most cultivated grain in Bangladesh. The production of wheat is increasing day by day in this country. The total area for wheat cultivation now extends to about 1.78 lakh ha and the annual production is about 10 lakh m tons (BBS, 2017). The country requires about 40 lakh m tons of wheat seed annually. About 20,000 m tons of seed is supplied from the public sector and the rest (80,000 m tons) comes from the farmers (Department of Agricultural Extension, Bangladesh). Wheat plants are suffered from several diseases; and in 2016 wheat blast caused by *Magnaporthe oryzae* Pathotype *triticum* (Mot), a devastating disease of wheat was spotted in Bangladesh for the first time, the first case in Asia and confirmed with genome sequencing by Dr. Sophien Kamoun, Sinsbury Laboratory, UK.

In 1985, wheat blast was first reported on wheat (*Triticum aestivum* L.) in Parana State Brazil (Maciel, 2011). It has since spread throughout many of the important wheat producing areas of Brazil and to neighboring South American countries including Bolivia and Paraguay, then in Kentucky, USA, in 2011. It is now a serious production constraint for wheat in the tropics and sub tropic regions, including Brazil, Argentina, Bolivia and Paraguay causing yield losses of up to 100% (Peng et al., 2011).

The total area of wheat cultivation in Bangladesh in 2016 was about 498,000 ha (Department of Agricultural Extension, Bangladesh). Wheat blast was observed in the year 2016 at eight south-western districts viz., Pabna, Kushtia, Meherpur, Chuadanga, Jhenaidah, Jessore, Barisal and Bhola in Bangladesh (Malaker et al., 2016). Out of a 101,660 ha of cultivated wheat in those eight districts, an estimated 15% were affected by wheat blast. The severity of wheat blast and associated yield losses varied among districts. The highest percentage of infected wheat fields was observed in Meherpur (70%). Yield losses in different affected districts also varied. The highest average yield loss was recorded in Jhenaidah (51%). Although the average yield loss was lower than 51% across districts, yield losses in individual fields were as high as 100%. Importantly, 100% of government owned Bangladesh Agricultural Development Corporation (BADC) seed multiplications farm in the affected districts (ca. 355 ha) were completely cleared by burning to destroy pathogen inocula by the decision of the Ministry of Agriculture. Farmer wheat fields that were severely affected (up to 100%) were also burned (Islam et al., 2016).

From the couple of decades plant extracts are used for controlling different types of plant pathogenic fungi such as *Magnaporthe oryzae* causing rice blast, *Rhizoctonia solani* causing sheath blight of rice, *Bipolaris oryzae* causing brown spot of rice, *Bipolaris sorokiniana* on wheat leaves, *Phytophthora infestans* causing late blight of potato etc. Khanzada and Shah (2012) described the antifungal effect of the extracts of Garlic (*Allium sativum* L.), Neem (*Azadirachta indica* L.) and Calatropis (*Calotropis procera* L.) against *M. oryzae* by food poisoning method and only higher dose of garlic extract completely inhibited the mycelial growth of the test fungus. Garlic extract gave the best result in controlling seed-borne fungal pathogens and enhance seed germination of rice following Neem leaf extract (Riazuddin et al., 2009). Allamanda (*Allamanda cathertica*) leaves are the source of many compounds with medicinal properties and found promising antifungal effect (Rumana, 2004). Ashrafuzzaman and Khan (1992) evaluated the effects of the extract of Allamanda leaf (*A. cathertica*), Mehendi leaf (*Lawsonia alba*) and Duranta (*Duranta plumeiri*) inhibit mycelial growth and sclerotial formation of *Rhizoctonia solani*. Roy et al. (2013) reported that five plants extracts viz. Garlic tablet, Allamanda tablet, Neem leaf extract, Biskatali leaf extract and Zinger rhizome extract were assessed as seed treating agents against seed borne pathogen of jute.

Some fungicides can be used for controlling wheat blast. The frequent use of fungicides on crops may cause hazards to human beings, plant health, beneficial micro-organisms and develop fungicide resistance into the pathogens and residual toxicity in plant parts. On the other hand, some botanical pesticides and bio-control agents have proved to be most secure and have no adverse impact on environment (Iftikhar et al., 2010; Babar and Khan, 1999). As well as, use of chemical fungicides for controlling this disease might have health hazard for human being and animals. Therefore, environment-friendly management of this pathogen with botanical extracts will be very effective until the development of resistance cultivars against this pathogen in Bangladesh. As no work is done for controlling wheat blast disease by using plant extracts, and due to that this work is a new effort for non-chemical as well as environment friendly management of wheat blast in Bangladesh.

The present research was undertaken to find out the antifungal effect of some botanical extracts on Mot and to determine the effect of the plant extracts on disease reduction and yield contributing parameters of wheat.

## 2 Materials and Methods

The experiment was conducted in two phases namely laboratory and pot experiment from August, 2016 to March, 2017 in the laboratory and experimental field of Plant Pathology Department, Bangladesh Agricultural University, and Plant Pathology Division, Bangladesh Institute of Nuclear Agriculture.

Table 1. Specification of different treatments

| Treatments | Type | Plant part | Antimicrobial compounds |
|---|---|---|---|
| Neem leaf extracts (1:10) ($T_1$) | Botanical | Leaf | Azadirachtin |
| Bishkatali leaf extracts (1:10) ($T_2$) | Botanical | Leaf | Sesquiterpene dialdehydes polygodial, Warburganal |
| Nishinda leaf extracts (1:10) ($T_3$) | Botanical | Leaf | Hexadecanoic acid, Caryophyllene oxide. |
| Allamonda leaf extracts (1:10) ($T_4$) | Botanical | Leaf | Hexanoicacid, Octanoic acid |
| Acasia leaf extracts (1:10) ($T_5$) | Botanical | Leaf | 1,1-diphenyl-2-picrylhydrazyl (DPPH), Teracacidin |
| Tulsi leaf extracts (1:10) ($T_6$) | Botanical | Leaf | Methyl chavicol, Linalool |
| Mehendi leaf extracts (1:10) ($T_7$) | Botanical | Leaf | Anthroquinone |
| Datura leaf extracts (1:10) ($T_8$) | Botanical | Leaf | 2beta-(3,4-dimethyl-2,5-dihydro-1H-pyrol-2-yl-1- methylethyl pentonate |
| Bishkachu leaf extracts (1:10) ($T_9$) | Botanical | Leaf | Alocasin |
| Black cumin seed extracts (1:10) ($T_{10}$) | Botanical | Seed | Thymoquinone, Thymohydroquinone |
| Garlic clove extracts (1:10) ($T_{11}$) | Botanical | Clove | Allicin |
| Mehogoni seed extracts (1:10) ($T_{12}$) | Botanical | Seed | 3B-acetoxy-mexicanolide,6-acetyl-swietenine |
| Provax (0.2%) ($T_{13}$) | Chemical | – | Provaxaltonin |
| Nativo (0.2%) ($T_{14}$) | Chemical | – | Trifloxystrobin+ Tebuconazole |
| Control ($T_0$) | Only PDA | – | - |

## 2.1 Antifungal experiment with plant extracts

Fourteen treatments were used in this antifungal experiment which are mentioned in the Table 1.

### 2.1.1 Plant extracts and fungicidal solution preparation

Plant extracts were prepared at 1:5 ratios of plant materials and water. About 20 g of plant parts of each of them were chopped into small pieces. Water (100 ml) was added to chopped sample pieces and then blended. Then the material was filtered through the cotton cloth. Volume of 100 ml extracts of each treatment was made and then the extract was taken in a conical flask. Two chemical *viz*. Provax and Natovo were used in this *in vitro* experiment and the 0.2% solution of Provax was prepared by adding 2 g of fungicide in 1000 ml distilled water in an 1 L volumetric flask. After that the content within the flask was stirred properly with the help of magnetic stirrer. By the same way, 0.2% solution of Nativo was also prepared.

### 2.1.2 PDA plate containing plant extracts preparation

For this preparation 200g of clean peeled slice potato tubers were boiled in 500 ml of water. After that water was separated from boiled potato slices. 20g Dextrose and 15g Agar was added to separate boiled water and made the final volume 500 ml of double strength PDA medium. Then, 100 ml of plant extracts (1:5) was mixed with 100 ml of double strength PDA to prepare 200 ml volume of PDA medium containing plant extract (1:10). Then the PDA medium containing plant extracts was autoclaved and poured into petridishes for the preparation of PDA plate supplemented with plant extracts for subsequent study.

### 2.1.3 PDA plate inoculation with *M. oryzae* Pathotype *triticum* isolate and mycelial growth rate measurement

PDA plates supplemented with different plant extracts were inoculated with 5mm fungal blocks collected from pure culture of MoT and these blocks were transferred to the center of the petri plates with the help of sterilized needle. Ten (10) days after inoculation, the mycelial growth of MoT were recorded by measuring the average of two diameters at right angles to one another. Three replications were maintained for each of the treatment and the mean radial mycelial growth was considered for each of the treatment. Then the effect of plant extract was calculated as percent growth inhibition using the following formula as adopted by Satish et al. (2007) and Dubey et al. (2009).

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

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

## 2.2 Pot experiment

BARI Gom 25, one of the high yielding variety of wheat in Bangladesh was used for the pot experiment. This variety is also susceptible to blast disease.

### 2.2.1 Preparation of experimental pot and sowing of seed

Each of the plastic pot filled up with10 kg soil which was silt-loamy in nature. In total, 28 pots were prepared for 7 treatments including control and each of the treatment maintained with 4 replications. Twenty (20) seeds in each pot were sown in 15 November, 2016 in each of the prepared pot. Thinning was done at 30 days after sowing (DAS) to maintain 10 plants per pot. The fertilizers were applied in each pot as per recommended dose of the Fertilizer Recommendation Guide (BARC, 2012). Weeding was uniformly done in all the pots as per requirement. Irrigation was applied time to time when required.

### 2.2.2 Treatments for pot experiment

The following seven treatments including control were used to know the effect of plant extracts on the disease incidence and severity of wheat blast and different yield contributing parameters of wheat–

$T_1$ Garlic clove extract (1:10)
$T_2$ Black cumin seed extract (1:10)
$T_3$ Dhutara leaf extract (1:10)
$T_4$ Tulsi leaf extract (1:10)
$T_5$ Mehendi leaf extract (1:10)
$T_6$ Nativo (0.2%)
$T_7$ Control (Water)

### 2.2.3 Application of plant extracts and fungicidal solution

Plant extracts and fungicide were sprayed as solution on the plant surface as per treatments. Spraying was done at four different growth stages namely seedling stage, tillering stage, before emerging of ear and after emergence of ear. Adequate precautions were taken to avoid drifting of spray materials from one pot to the neighboring ones.

### 2.2.4 Inoculation of the growing plants

One day after application of the plant extracts and chemical, the treated plants were inoculated with mycelial suspension of MoT. Ten days old culture of MoT was used for the preparation of mycelial suspension with distilled water at 1:10 ratio (fungal mycelia: water). Then the filtered mycelial suspension was sprayed on the foliar parts of the treated plants with the help of a hand sprayer at four different growth stages mentioned earlier. After inoculation all the plots were covered with polythene sheet keeping small holes on the sheet.

### 2.2.5 Disease incidence and severity assessment

Incidence of wheat blast disease was calculated in each pot according to following formula developed by Rajput and Bartaria (1995).

$$\text{Disease incidence (\%)} = \frac{P_i}{P_t} \times 100 \qquad (2)$$

where $P_i$ = Number of panicle infected, and $P_t$ = Total number of panicle.

On the other hand, disease severity was determined by observing disease symptoms on wheat spikes and assessment of severity was done by using 10 classes according to Maciel et al. (2013).

### 2.2.6 Crop harvesting and data collection

The crop was harvested on 28 February, 2017 at full ripening stage and data on the yield and yield contributing parameters were collected.

## 2.3 Data analyses

The experiment was conducted in a completely randomized design (CRD) with four replicates. Statistical analysis for phenotypic and yield data were carried out with SPSS statistical software.

# 3 Results

## 3.1 Isolation, identification and development of pure culture of *M. oryzae* Pathotype *triticum*

After collection, the diseased plant samples were brought in to the laboratory and preserved in the refrigerator for isolation. Later on the pathogen were isolated from infected wheat spikes which were collected from farmer's field. Standard blotter method (ISTA, 1996) was used for the isolation of this MoT pathogen from infected spikes. In this blotter method, 10 spikes were placed on blotter paper (moistened with water drop) in plastic petridishes. Then the petridishes were incubated at the incubation room at 25 °C. After 3-4 days of incubation pathogenic structures (mycelia) on spikes and blotter paper were observed under stereo binocular microscope. Then the fungal structures were transferred on Potato Dextrose Agar (PDA) medium for 10-12 days in the incubation room to allow the fungus to grow. The concern pathogen was detected by preparing slide and comparing the morphological character as pear shaped conidia (Fig. 1).

However, the fungal block was transferred to the centre of a fresh PDA plate using a sterilized block

Table 2. Colony characters of M. oryzae Pathotype triticum on PDA media supplemented with different plant extracts

| Treatments | Colony characters | | |
|---|---|---|---|
| | Substrate/media color | Colony color | Margin of colony |
| Potato dextrose agar (PDA) = $T_0$ | Light brownish | Grey ash centre and black margin | Regular |
| Neem leaf extract + PDA = $T_1$ | Green | White | Regular |
| Bishkatali leaf extract + PDA = $T_2$ | Green | White | Regular |
| Nishinda leaf extract + PDA = $T_3$ | Light green | White | Regular |
| Alamonda leaf extract + PDA = $T_4$ | Light green | White | Regular |
| Acasia leaf extract + PDA = $T_5$ | Dark green | White | Regular |
| Tulsi leaf extract + PDA = $T_6$ | Red | Black | Regular |
| Mehendi leaf extract + PDA = $T_7$ | Red | Black | Regular |
| Datura leaf extract + PDA = $T_8$ | Dark Green | Black | Regular |
| Bishkachu leaf extract + PDA = $T_9$ | Lemon | Ashy center with white center | Regular |
| Black cumin seed extract + PDA = $T_{10}$ | Black | Black | Regular |
| Garlic clove extract + PDA = $T_{11}$ | Light Yellow | Black | Regular |
| Mehogoni seed extract + PDA = $T_{12}$ | Orange | White | Regular |
| Provax (0.2%) = $T_{13}$ | Blue | Black | Regular |
| Nativo (0.2%) = $T_{14}$ | Creamy White | Black | Regular |

cutter. The plates were sealed with parafilm and all the isolates then grown into the incubator at 25 °C with alternate 12/12 h UV light and darkness for 10 days. Sequential culturing from fungal stock was done for 4-6 times to get a pure culture which was used for inoculation (Fig. 4). Inoculation was done under laminar air-flow cabinet.

### 3.2 Growth characteristics of *M. oryzae* Pathotype *triticum* on PDA media supplemented with different plant extracts

The growth characteristics like colour of substrate/media, color of colony and margin of colony of MoT on PDA media supplemented with different plant extracts (1:10) were observed in this study. The color of the colony of MoT having grey ash centre and black margin in case of PDA media. Whereas, PDA media supplemented with Neem leaf extraxt, Nishinda leaf extract, Allamonda leaf extract, Bishkatali leaf extract, Acasia leaf extract and Mehogoni seed extract showed white color colony and PDA media supplemented with Tulsi leaf extract, Mehendi leaf extract, Datura leaf extract, Garlic clove extract, Black cumin seed extract showed black color colony. PDA media supplemented with Bishkochu leaf extract showed colony with ashy center and white margin. This result indicated that PDA media supplemented with different plant extracts have an impact on the colony color of MoT (Fig. 2; Table 2). In addition, PDA media containing 0.2% fungicides such as nativo and provex showed no fungal colony after inoculation with MoT. On the other hand, margin of the pure colony of MoT on PDA media and PDA supplemented with different plant extracts showed mostly regular form (Fig. 2; Table 2).

### 3.3 Effect of different plant extracts on percent growth inhibition of *M. oryzae* Pathotype *triticum*

Effect of twelve selected plant extracts at 1:10 ratio on the percent growth inhibition of MoT were also observed in this study. The result showed that there was no growth inhibition occurred on control plate. The minimum percent growth inhibition (7.5%) was recorded in plates containing Allamonda leaf extract compared to control while the maximum inhibition was recorded in the plates containing Garlic clove, Tulsi leaf, Mehendi leaf (93.75%) and Black cumin seed extract (90%) respectively (Fig. 3, Table 3) after 10 days of inoculation.

### 3.4 Effect of plant extracts on the disease incidence and severity of wheat blast disease

Effect of five selected plant extracts based on their efficacy in *in vitro* experiment, were evaluated for disease incidence and severity of wheat blast in the pot. The minimum percentage of disease incidence and severity of treated plants were recorded in case of Garlic clove extract 16.28% and 3.5% followed by Nativo treated plants and maximum percentage of

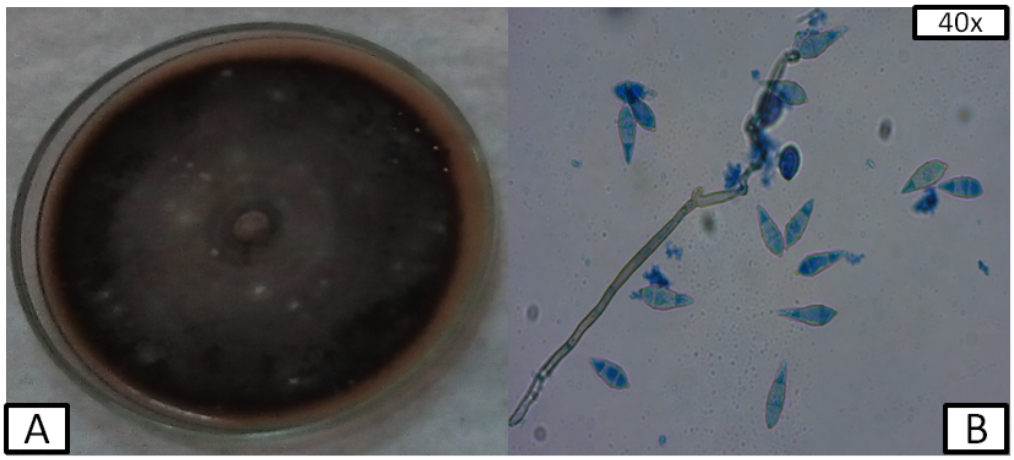


Figure 1. (A) Pure culture, and (B) different structures of *Magnaporthe oryzae* Pathotype *triticum*.

disease incidence and severity was recorded in case of control (98.11 and 100%) (Table 4).

### 3.5 Effect of plant extracts on plant height, ear length, number of healthy and diseased ear/pot of wheat

Five selected plant extracts were used to know the effect of botanicals on the yield contributing attributes of wheat in inoculated and control plants. The maximum height (92.70 cm) of wheat plant was recorded in case of Mehendi leaf extract and the minimum plant height (86.54 cm) was found in case of Datura leaf extract. The maximum ear length was 9.20 cm for Garlic clove extract and minimum ear length was recorded for Tulsi (7.94 cm). Number of total ear/pot ranged from 10.75 to 13.25 for Mehendi leaf and Garlic clove and Black seed extract respectively. Number of healthy ear/pot ranged from 4.5 to 13 for Tulsi leaf and Garlic clove extract respectively. Number of diseased ear/pot ranged from 0.25 to 7.50 for garlic clove and Tulsi leaf extract respectively. In case of number of total, healthy and diseased ear/pot, garlic clove extract ( 13.25, 13.00 and 0.25) sprayed plants showed better results compared to other plant extracts and water sprayed control (11.5, 7.5 and 4.00) showed the lowest value. On the other hand, chemical i.e. Nativo sprayed plants showed very much similar results for these yield attributes that showed by Garlic sprayed pot (Table 5).

### 3.6 Effect of foliar spray of plant extracts on number of healthy and diseased spikelts/ear, number of healthy and diseased grains/ear of wheat

Number of total spikelets/ear ranged from 34.20 to 14.40 in case of Garlic clove and Black cumin seed extract respectively. Number of healthy spikelets /ear ranged from 33.40 to 5.80 for garlic clove and control and number of diseased spikelets/ear from 0.80 to 23.60 for Garlic clove extract and control respectively. On the other hand number of total, healthy and diseased grains/ear ranged from 23.00 to 8.40, 21.80 to 0.00 and 2.20 to 8.40 Garlic clove extract and water sprayed control respectively. In case of number of total, healthy and diseased spikelets/ear, Garlic clove extract (34.20, 33.80 and 0.80) sprayed pot showed better results where water sprayed control (29.40, 5.80 and 23.60) showed the lowest value. On the other hand the number of total, healthy and diseased grains/ear Garlic (23.00, 21.80 and 2.20) sprayed pot showed better results compared to other plant extract and water sprayed control (8.40, 0.00 and 8.40) showed the lowest value. Nativo sprayed pot showed very much similar results that showed by Garlic sprayed pot (Table 5). Grains from blast-infected heads were small, shriveled, deformed, and had low test weight (Fig. 4).

### 3.7 Effect of plant extracts on weight of healthy and diseased grains/ear and weight of 1000 grains of wheat

Weight of total, healthy and diseased grains/ear ranged from 4.72 g to 1.80 g, 4.20 g to 0.00 g and 0.52 to 1.80 g. On the other hand the weight of 1000 seeds ranged from 56 to 37.4 g for Garlic clove extract and water sprayed control respectively. In case of weight of total, healthy and diseased grains/ear, Garlic clove extract sprayed plants showed better results (4.72, 4.20 and 0.52 g) followed by Nativo (4.6, 4.3 and 0.3 g) where water sprayed control (1.80, 0.00 and 1.80 g) showed the lowest value. On the other hand the weight of 1000 seeds Garlic clove extract (56 g) sprayed pot showed better results followed by Nativo (54 m) where water sprayed control (37.40 g) showed the lowest value.

Table 3. Mycelial growth and percent mycelial growth inhibition of *M. oryzae* Pathotype *triticum* in plant extracts supplemented PDA media at 10 DAI

| Treatment | Mycelial growth (mm) | Mycelial growth inhibition (%) |
|---|---|---|
| Control = $T_0$ | 80.00a | – |
| Neem leaf extract = $T_1$ | 66.50c | 16.88 |
| Bishkatali leaf extract = $T_2$ | 63.50d | 20.63 |
| Nishinda leaf extract = $T_3$ | 56.33e | 29.59 |
| Alamonda leaf extract = $T_4$ | 74.00b | 7.50 |
| Acasia leaf extract = $T_5$ | 58.67e | 26.66 |
| Tulsi leaf extract = $T_6$ | 5.00i | 93.75 |
| Mehendi leaf extract = $T_7$ | 5.00i | 93.75 |
| Datura leaf extract = $T_8$ | 5.00i | 93.75 |
| Bishkachu leaf extract = $T_9$ | 50.17f | 37.29 |
| Black cumin seed extract = $T_{10}$ | 8.00h | 90.00 |
| Garlic clove extract = $T_{11}$ | 5.00i | 93.75 |
| Mehogoni seed extract = $T_{12}$ | 43.50g | 45.63 |
| Provax (0.2%) = $T_{13}$ | 5.00i | 93.75 |
| Nativo (0.2%) = $T_{14}$ | 5.00i | 93.75 |
| LSD(0.05)‡ | 2.506 | |

‡ LSD = Least significant difference @ 5% level of significance. In a column, means followed by same letter(s) are statistically similar at 5% level by DMRT.

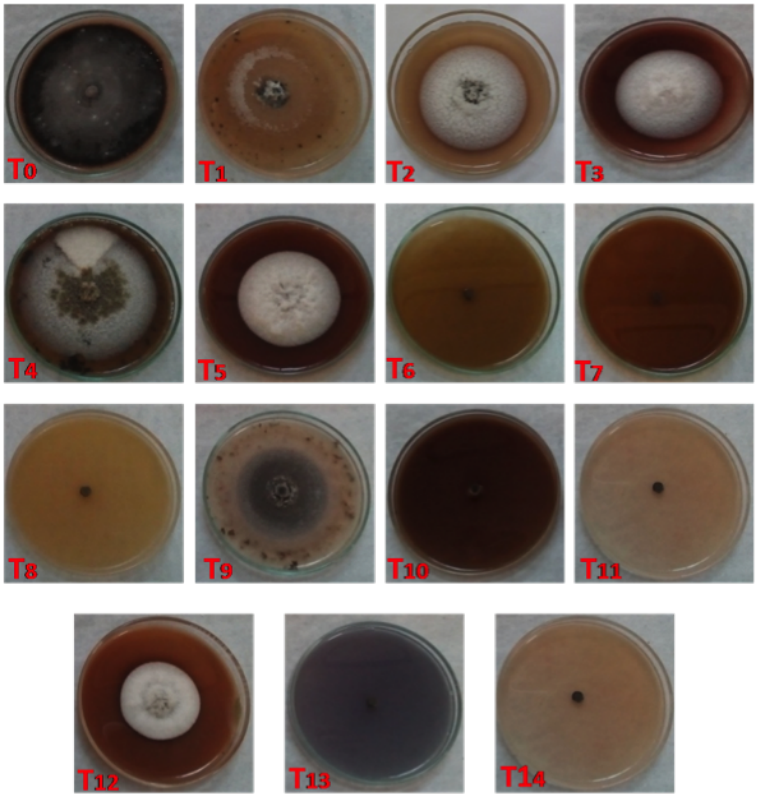


Figure 2. Growth status, colour and appearance of pure colonies of MoT on PDA media supplemented with different plant extracts (10 Days after inoculation) (Here,$T_0$ = Potato dextrose agar, $T_1$ = PDA + Neem leaf extract, $T_2$ = PDA + Bishkatali leaf extract, $T_3$ = PDA + Nishinda leaf extract, $T_4$ = PDA + Allamonda leaf extract, $T_5$ = PDA + Acasia leaf extract, $T_6$ = PDA + Tulsi leaf extract, $T_7$ = PDA + Mehendi leaf extract, $T_8$ = PDA + Datura leaf extract, $T_9$ = PDA + Bishkochu leaf $T_{10}$ = PDA + Black cumin seed extract, $T_{11}$ = PDA + Garlic clove extract, $T_{12}$ = PDA + Mehogoni seed extract, $T_{13}$ = PDA + Provax 0.2%, $T_{14}$ = PDA + Nativo 0.2%).

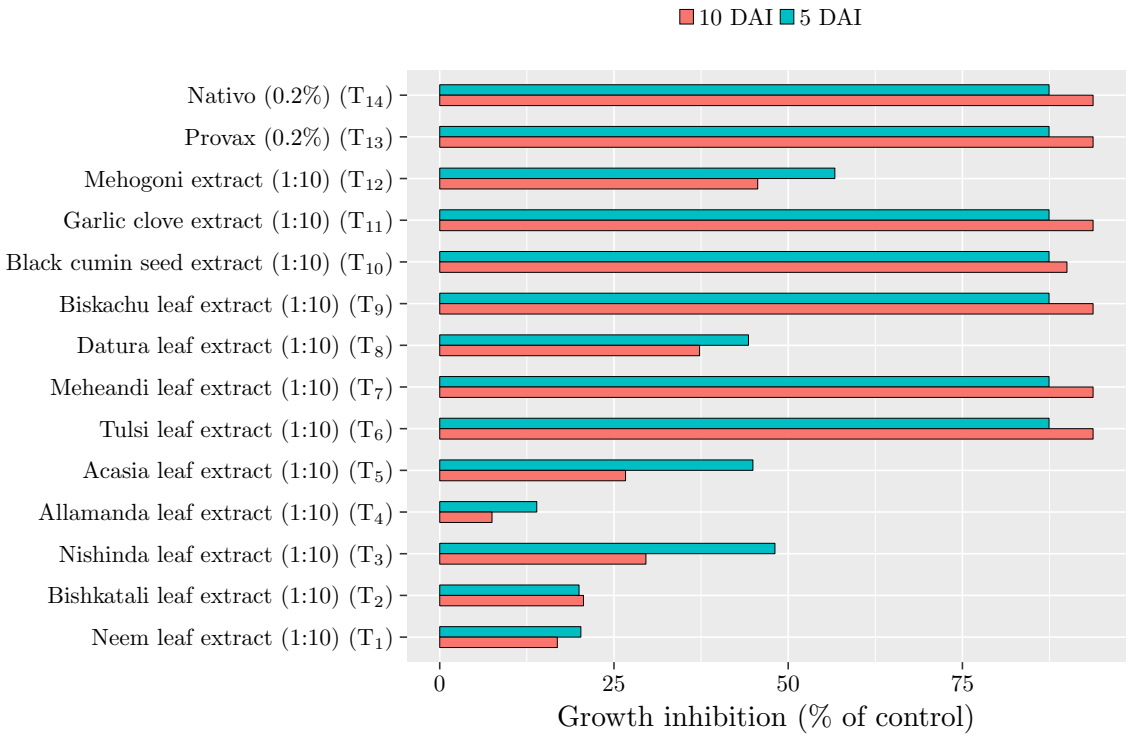


Figure 3. Percent growth inhibition of *M. oryzae* Pathotype *triticum* on PDA plate containing plant extracts (1:10) at 5 and 10 days after inoculation (DAI) separately over control.

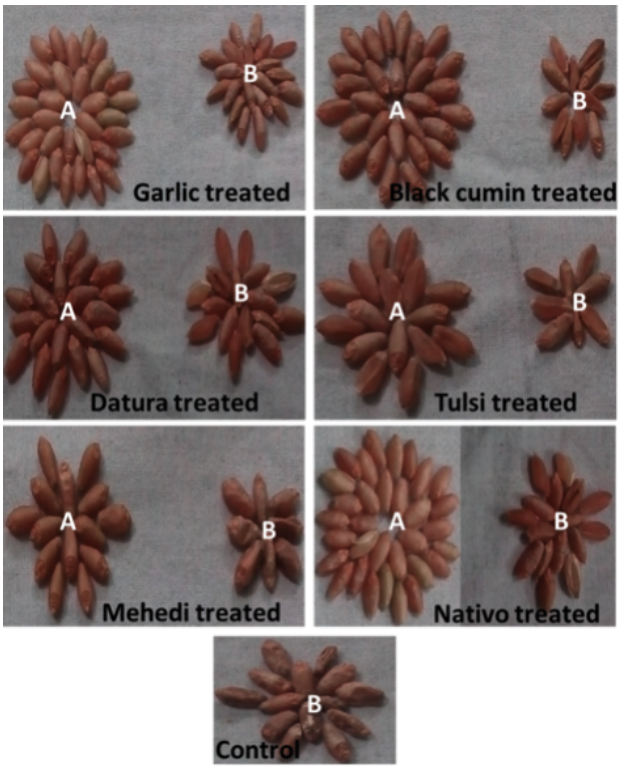


Figure 4. Efficacy of plant extracts on the number of healthy and infected grains/ear of wheat. A = healthy, and B = infected grains

Table 4. Evaluation of disease incidence and severity percentage of wheat blast

| Treatments | Disease incidence (%) | Disease severity (%) |
|---|---|---|
| Garlic clove extract (1:10) = $T_1$ | 16.28 | 3.5 |
| Black cumin seed extract (1:10) = $T_2$ | 34.78 | 7.5 |
| Datura leaf extract (1:10) = $T_3$ | 51.92 | 44 |
| Tulsi leaf extract (1:10) = $T_4$ | 62.5 | 57.5 |
| Mehedi leaf extract (1:10) = $T_5$ | 66 | 68 |
| Nativo (0.2%) = $T_6$ | 15.2 | 3.5 |
| Control = $T_0$ | 98.11 | 100 |

Table 5. Efficacy of foliar spray of plant extracts on grain yield and yield contributing characters, and grain and ear health of wheat

| Parameter | Treatment† | | | | | | | LSD(0.05)‡ |
|---|---|---|---|---|---|---|---|---|
| | $T_1$ | $T_2$ | $T_3$ | $T_4$ | $T_5$ | $T_6$ | $T_0$ | |
| Plant height (cm) | 86.92e | 87.45d | 86.54e | 88.32c | 92.70a | 87.60d | 91.43b | 0.2028 |
| Ear length (cm) | 9.20a | 8.40b | 8.40b | 7.94c | 9.06a | 9.1a | 8.30b | 0.3081 |
| No. of ear/pot | 13.25a | 13.25a | 12.75b | 12b | 10.75d | 12.80b | 11.5c | 0.3132 |
| No. of healthy ear/pot | 13.00a | 10.25b | 5.50e | 4.5f | 9.00c | 12 | 7.5d | 0.2578 |
| No. of diseased ear/pot | 0.25d | 3.00bc | 7.25a | 7.50a | 1.75cd | 0.80d | 4.00b | 1.995 |
| No. of spikelets/ear | 34.20a | 14.40f | 19.80d | 18.20e | 26.00c | 33.80a | 29.40b | 0.2869 |
| No. of healthy spikelets/ear | 33.40a | 12.40b | 7.00d | 6.00e | 11.20c | 33.30a | 5.80f | 0.2869 |
| No. of diseased spikelets/ear | 0.80c | 2.00c | 12.80b | 12.20b | 14.80b | 0.50c | 23.60a | 2.546 |
| No. of grains/ear | 23.00a | 13.40c | 14.40b | 11.40d | 10.60e | 22.40a | 8.40f | 0.2028 |
| No. of healthy grains/ear | 21.80a | 9.40c | 8.60d | 3.00e | 3.80e | 19.50b | 0.00f | 1.172 |
| No. of diseased grains/ear | 2.20e | 4.00d | 5.80c | 8.40a | 6.80b | 2.90e | 8.40a | 0.3328 |
| weight of grains/ear (g) | 4.72a | 3.95b | 3.15c | 2.59d | 2.58d | 4.6a | 1.80e | 0.943 |
| weight of healthy grains/ear (g) | 4.20a | 2.60b | 1.95bc | 0.95cd | 0.73cd | 4.3a | 0d | 0.2578 |
| weight of diseased grains/ear (g) | 0.52b | 1.35ab | 1.20ab | 2.00a | 1.85a | 0.3b | 1.80a | 0.5726 |
| weight of 1000 grain (g) | 56b | 53.6a | 50a | 46.5a | 45a | 54b | 37.4a | 0.2808 |

† $T_1$ = Garlic clove extract (1:10), $T_2$ = Black cumin seed extract (1:10), $T_3$ = Datura leaf extract (1:10), $T_4$ = Tulsi leaf extract (1:10), $T_5$ = Mehedi leaf extract (1:10), $T_6$ = Nativo (0.2%), $T_0$ = Control.
‡ LSD = Least significant difference @ 5% level of significance. In a column, means followed by same letter(s) are statistically similar at 5% level by DMRT.

## 4 Discussion

Blast disease caused by MoT is a new threat to wheat production which can causes yield losses up to 100% (Peng et al., 2011). In 2016, it was first time appeared at eight different districts of south-western region in Bangladesh, which results in burning the total fields in most of the cases. So, management of wheat blast is very important for the sustainable production of wheat in Bangladesh. Some chemical fungicides are used for controlling wheat blast, but those chemicals are harmful for human being as well as for the environment. The frequent use of fungicides on crops may cause hazards to human beings, plant health, beneficial micro-organisms, and developed fungicidal resistance into the pathogens and residual toxicity in plant parts. On the other hand, some botanical pesticides and bio-control agents have proved to be most secure and have no adverse impact on the environment (Iftikhar et al., 2010; Babar and Khan, 1999). So, finding out of eco-friendly and non-toxic approaches for wheat blast management is the main aspect of the present research work.

This experiment had two phases namely laboratory experiment and pot experiment. In the laboratory, the efficacy of 12 plant extracts 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 were evaluated against MoT. The effect of plant extracts on radial mycelial growth (mm) of MoT showed that four plant extracts *viz*. Tulsi leaf extract, Mehendi leaf extract, Datura leaf extract and Garlic clove extract inhibit the highest percentage (93.75%) of mycelial growth followed by Black cumin seed extracts (90%) at 10 DAI where Allamonda leaf extract showed lowest percentage

of mycelial growth inhibition (7.5%) over control. This result is in conformity with the findings where they described that at the 10,000 ppm concentration of H. anthelminthicus fruit extracts exhibited antifungal potential to growth inhibition, and recorded 100% growth inhibition against *Pyricularia oryzae*, *P. palmivora* and *R. solani* followed by *S. rolfsii* at 96.33% when compared with water control. *X. lanceatum* fruit extract logged excellent inhibitory activity against *P. oryzae* (Jantasorn et al., 2016). There is an alternative measure to control rice blast disease by using leaf extract of *Piper caninum* blume. Antifungal activity of *P. caninum* against*P. oryzae* was done under laboratory condition on potato dextrose agar (PDA) medium and the leaf extract of *P. caninum* significantly ($P<0.05$) inhibited the fungal radial growth, spores formation, and biomass formation (Suriani et al., 2015). Aqueous leaf extract of *Azadirachta indica*, *Emblica officinalis*, *Pongamia glabra* and *Acacia nilotca* inhibit the mycelial growth of *Magnaporthe oryzae* causing leaf blast and *Bipolaris oryzae* causing brown spot in rice under laboratory condition (Pandey, 2015).

Based on the results of the *in vitro* experiment, five best antifungal plant extracts (Garlic, Black cumin, Datura, Tulsi and Mehendi) have been used for the control of blast disease in the pot within net house condition. However, the plant extracts were used in the pot experiment to evaluate the efficacy of those extracts on disease incidence and severity and some yield contributing parameters in treated and control plants.

The results of the pot experiment clearly showed that the minimum percentage of disease incidence and severity of treated plants were recorded in case of Garlic clove extract 16.28% and 3.5% followed by Black cumin seed extract 34.78% and 7.5%, respectively and maximum percentage of disease incidence and severity was recorded in case of control (98.11 and 100%). However, these findings are in agreement with the findings reported by other researchers. Eupatorium (*Chromolaena odorata* L.) an obnoxious weed which can inhibit the growth of *Pyricularia oryzae* when eupatorium extract extracted with acetone (91.3%) followed by methanol (85.6%), distilled water (74.5%) and petroleum ether (53.9%). The results of field trail also indicated that among different extracts, the PDI of leaf blast was lowest in 15% acetone extract (23.8) and the distilled water extract (5 to 15%) indicating its effectiveness in controlling blast disease of rice (Manjappa, 2013). Flora and Rani (2013) reported that the foliar application of aqueous concentrate of *Stoechospermum marginatum* reducing the severity of fungal blast of rice caused by *Pyricularia oryzae* was investigated.

The present study also showed 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 visible differences in inoculated and control plants after treating with different botanical extracts. The maximum height (92.70 cm) of wheat plant was recorded in case of Mehendi leaf extract and the minimum plant height (86.54 cm) was found in case of Datura leaf extract. The maximum ear length (9.20 cm) was recorded for Garlic clove extract and minimum ear length was recorded for Tulsi (7.94 cm). Number of total ear/pot ranged from 10.75 to 13.25 for Mehendi leaf and Garlic clove extract, respectively. Number of healthy ear/pot ranged from 4.5 to 13 for Tulsi leaf and Garlic clove extract, respectively. Number of diseased ear/pot ranged from 0.25 to 7.50 for Garlic clove and Tulsi leaf extract. In case of number of total, healthy and diseased ear/pot, Garlic clove extract ( 13.25, 13.00 and 0.25) sprayed plants showed better results compared to other plant extracts. The pot experiment also explained that the number of total, healthy and diseased spikelets/ear and number of total, healthy and diseased grains/ear of wheat were differed in case of the plants treated with different plant extracts. Yield of spikelets and grain was also varied significantly in different treatments. Garlic clove extract showed best result as the highest production of total, healthy and diseased spikelets/ear (34.20, 33.40 and 0.80, respectively) The highest number of total grain/ear, healthy grains/ear and the maximum weight for total grains/ear, healthy grains/ear and 1000 grains/ear also found in garlic clove extract treated plants followed by other plant extract. These findings are also corroborates the findings of earlier researchers. The effects of *Aloe vera*, *Allium sativum*, *Annona muricata*, *Azadirachta indica*, *Bidens pilosa*, *Camellia sinensis*, *Chrysanthemu coccineum*, processed *Coffee arabica*, *Datura stramonium*, *Nicotiana tabacum* and *Zingiber officinalis* extracts for control of rice blast disease (*Pyricularia grisea*) both in-vitro and in-vivo were evaluated. The results indicate that processed *C. arabica* at 10% and 25% (*v*/*v*) had the highest (81.12%) and (89.40%) inhibitory effect, respectively, against *P. grisea*. These plant extracts can be used for rice seed treatment to manage rice blast disease (Hubert et al., 2015). Roots of *Chloranthus japonica* and stem of *Paulownia coreana* were effective in the management of rice blast. Treatments with *P. guineense* and Carbendazim had comparable for leaf blast suppression effects (Choi et al., 2004).

Based on the findings of the present study it may be concluded that garlic clove extract was most effective under in vitro as it completely inhibited mycelial growth up to 93.33% and exhibited minimum disease incidence and severity and highest yield contributing parameters at 1:10 dilution. From the literature it is clear that garlic extract contain antifungal compounds Allicin (Ankri and Mirelman, 1999; Harris et al., 2001; Borlinghaus et al., 2014) and this compound might also inhibit the vegetative and reproductive growth of MoT studied here. Moreover, garlic extract might

also trigger the genes which are involved with the induced resistance in the host plants (Ghazanfar et al., 2011; Satya et al., 2007).

Since wheat blast is the threat of wheat production in Bangladesh, ways of controlling the disease need to be developed resistant varieties are required to stabilize seed production and to promote sustainable agriculture without hazardous chemical control. But it's very difficult, laborious, costly and time consuming to developed a resistant variety against blast disease. Hence the introgression of environment-friendly management i.e. use of different botanical pesticide especially garlic based formulated bio-pesticide for controlling blast disease of wheat is the only viable way for the long term control of this disease and save the nature as well as getting balanced the environment from the hazardous effect of chemical fungicides.

## 5 Conclusion

Wheat blast is caused by *Magnaporthe oryzae* Pathotype *triticum* is a new disease in Bangladesh and caused up to 100% yield loss first time in some wheat growing areas of Bangladesh in the year 2016. As it is first time flares in Bangladesh no appropriate control measures have been developed till now. Moreover, this fungus strikes the heads of wheat and it is difficult for fungicide to reach. Therefore, environment-friendly management of this pathogen with botanical extracts or biological agents will be very effective until the development of resistance cultivars against this notorious pathogen in Bangladesh. Based on the findings of the present study it may be concluded those Garlic cloves extract was the most effective under in vitro as it completely inhibited mycelial growth up to 93.75%. In additions, garlic clove extract also effective for control of wheat blast disease as it showed minimum percentage of disease incidence and severity under pot experiment. However, this experiment with plant extracts needs to be coined out to assess the field efficacy of these botanical extracts either alone or in combination with different concentrations and frequencies in controlling blast of wheat.

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The Official Journal of the **Farm to Fork Foundation**
ISSN: 2518–2021 (print)
ISSN: 2415–4474 (electronic)
http://www.f2ffoundation.org/faa