

**EFFECTS OF SOME PLANT EXTRACTS ON CONTROLLING
WHEAT BLAST CAUSED BY *Magnaporthe oryzae* Pathotype
triticum IN BANGLADESH**

MS THESIS

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DECEMBER 2017

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A Thesis

**Submitted to
Bangladesh Agricultural University, Mymensingh
In Partial Fulfillment of the Requirements for the Degree of
Master of Science
In
Plant Pathology**

By

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EFFECTS OF SOME PLANT EXTRACTS ON CONTROLLING WHEAT BLAST CAUSED BY *Magnaporthe oryzae* Pathotype *triticum* IN BANGLADESH

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ABSTRACT

An experiment was conducted at the Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh and the Plant Pathology Division, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh. to evaluate the efficacy of twelve plant extracts viz. Neem (*Azadirachta indica*), Bishkatali (*Polygonum hydropiper*), Nishinda (*Vitex negundu*), Allamonda (*Allamanda cathartica*), Acasia (*Acacia auriculiformis*), Tulsi (*Ocimum tenuiflorum*), Mehendi (*Lametalsonia alba*), Datura (*Datura metel*), Bishkochu (*Alocasia forficata*), Black cumin (*Nigella sativa*), Garlic (*Allium sativum*), Mehogoni (*Swietenia macrophylla*) @ 1:10 along with two fungicides Provax and Nativo @ 0.2% as check against *Magnaporthe oryzae* Pathotype *triticum* in both two phases viz. laboratory and pot experiment. In the laboratory experiment, the efficacies of plant extracts were evaluated by measurement of percent inhibition of radial mycelial growth of *M. oryzae* Pathotype *triticum*. 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, whereas Allamonda leaf extract showed lowest percentage of mycelial growth inhibition (7.5%). On the basis of *in vitro* test five plant extracts were selected to conduct pot experiment to evaluate the blast incidence and severity and some yield contributing parameters in treated and control plants. Minimum percentage of blast 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 % of disease incidence and severity (66.0% and 68.0%). Garlic clove extracts also showed best performance for many yield contributing parameters like 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 gm) followed by Black cumin, whereas Mehendi leaf extract treated plants showed lowest value for all the yield contributing parameters among the plant extracts used in this experiment. Both in *in vitro* 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.

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CHAPTER I

INTRODUCTION

As an agricultural country different types of agricultural crops are grown in Bangladesh. Cereal crops are main cultivated crops here. After rice, wheat is the second most cultivated grain in Bangladesh. Wheat is a grass plant of the genus *Triticum* widely cultivated for its seed, a cereal grain which is a worldwide staple food. In the early 1990s, a World Bank study concluded that wheat production were so low and wheat was so unprofitable, compared with *boro rice*, that there was no good reason to encourage the expansion of wheat production in Bangladesh. By 1994s, however, the situation had changed. As Bangladesh neared self-sufficiency in rice, its profitability was declining. By then, the demand for wheat was increasing faster than the demand for rice, and imports of wheat were growing.

Now-a-days due to increasing demand of wheat, government takes necessary steps for contributing a lot to the wheat production. With the help of government to wheat production, different high yielding variety, proper price of the seeds and fertilizers, intensive care of Department of Agriculture and the suitable environment of Bangladesh for wheat cultivation; 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, the most important being rusts, smuts, black point, loose smut, powdery mildew and seedling blight etc. In 2016 wheat blast, 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, Sainsbury Laboratory, UK.

In 1985, wheat blast was first reported on wheat (*Triticum aestivum* L.) in Parana State Brazil (Igarashi *et al.* 1986; 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 in the tropics and sub tropic regions, including Brazil, Argentina, Bolivia and Paraguay causing yield losses of up to 100% (Penga *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 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).

Wheat blast is caused by *M. oryzae* Pathotype *triticum* although scientists are still debating its exact identity. After the genomic analysis of a huge amount of *Magnaporthe* strains scientists have proposed the name of this pathogen is *Pyricularia oryzae* (Castroagudin *et al.* 2016). *M. oryzae* causing wheat blast is a new pathogen in Bangladesh and owing to that we have to find out the proper control measures of this pathogen. Some fungicides can be used for controlling wheat blast. But, this fungus attacks the heads of wheat and it is difficult for fungicide to reach. 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 *et al.*, 2011). 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.

From the couple of decades plant extracts are used for controlling different types of plant pathogenic fungi such as *Pyricularia 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. Hajano *et al.* (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 cathartica*) leaves are the source of many compounds with medicinal properties and found promising antifungal effect (Romana, 2004). Asharafuzzaman and Khan (1992) evaluated the effects of the extract of Allamanda leaf (*Allamanda cathartica*) , Mehendi leaf (*Lawsonia alba*) and Duranta (*Duranta plumeiri*) inhibit mycelial growth and sclerotial formation of *Rhizoctonia solani*. Roy *et al.* (2011) 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. They also stated that garlic tablet was effective in controlling seed-borne fungal infection; consequently the seed germination was high. They also found that the effect of Allamanda tablet was similar to that of garlic tablet and mentioned that Neem leaf extract was able to reduce seed-borne fungi but the other three extracts were not effective in controlling

seed-borne infection. In their study they showed that the performance of garlic tablet was similar to that of Vitavax-200 and a significant increase in seedling vigor was also observed over untreated control after garlic treatment.

However, in sight of the above backgrounds, the present research work has been designed to achieve the following objective-

- To find out the antifungal effect of some botanical extracts on *Magnaporthe oryzae* Pathotype *triticum*.
- To determine the effects of the plant extracts on yield contributing parameters of wheat.

CHAPTER II

REVIEW OF LITERATURE

Wheat blast caused by *Magnaporthe oryzae* Pathotype *tritricum* is a recent problem in Bangladesh and caused enormous yield loss in the year 2016 at the South-East region of Bangladesh. Consequently literature on management practices especially control of *M. oryzae* with plant extracts is very limited in the world and there is no previous report on the management of wheat blast with plant metabolites until to date in Bangladesh. There are some related literatures on the wheat blast pathogen and the management of plant diseases with plant extracts are presented below.

2.1. Wheat blast disease

Kohli (2011) stated that wheat blast disease caused by *Pyricularia grisea* (telemorph *Magnaporthe grisea*), especially in the tropical parts of the Southern Cone Region of South America. This disease was first observed in 1985 in the State of Parana in Brazil, which is now an endemic disease in the low lying Santa Cruz region of Boliva, south and south-eastern part of Paraguay and central and southern Brazil in recent years.

Penga *et al.* (2011) reported that wheat blast now become a serious production constraint in the tropics and sub tropic regions, including Brazil, Argentina, Bolivia and Paraguay in recent years causing yield losses of up to 100%.

Silva *et al.* (2009) said that due to the lack of resistant cultivars and effective fungicides for disease management, wheat blast is widely distributed across all the wheat-cropping areas in Brazil, causing crop losses from 40–100 %.

Barea and Toledo (1996) stated that in 1996 blast was reported for the first time outside of Brazil, in Bolivia's most important region for wheat production, the Santa Cruz Department

2.2 Wheat blast disease in Bangladesh

Islam *et al.* (2016) described that in February 2016, a new fungal disease of wheat called wheat blast was observed for the first across eight southwestern districts of Bangladesh which has an epidemic spread to 15,000 hectares, about 16% of the cultivated wheat area in Bangladesh, with yield loss up to 100%. They also found that, the outbreak of wheat blast in Bangladesh was most likely caused by a wheat-infecting South American lineage of the blast fungus *Magnaporthe oryzae*.

Malakar *et al.* (2016) reported that in the 2016, a wheat blast outbreak was reported for the first time outside of South America, in Bangladesh's districts of Kushtia, Meherpur, Chuadanga, Jhenaidah, Jessore, Barisal, Bhola, Magura, Narail, and Faridpur.

2.3. Wheat blast pathogen, *Magnaporthe oryzae* Pathotype *triticum*

Farman *et al.* (2017) described that the comparative genome analyses showed that fungal isolates from diverse wheat regions in Bangladesh appeared clonal and were closely related to highly aggressive MoT isolates from South America.

Castroagudin *et al.* (2016) stated that *Pyricularia oryzae* is a species complex that causes blast disease on more than 50 species of poaceous plants. *Pyricularia oryzae* has a worldwide distribution as a rice (*Oryza*) pathogen and in the last century emerged as an important wheat (*Triticum*) pathogen in southern Brazil. Presently, *P. oryzae* pathotype *Oryza* is considered the rice blast pathogen, whereas *P. oryzae* pathotype *Triticum* is the wheat blast pathogen. In this study they investigated whether the *Oryza* and *Triticum* pathotypes of *P. oryzae* were distinct at the species level. They also describe a new *Pyricularia* species causing blast on several other poaceous hosts in Brazil, including Wheat and they conducted a phylogenetic analyses using 10 housekeeping loci from an extensive sample ($N = 128$) of sympatric

populations of *P. oryzae* adapted to rice, wheat and other poaceous hosts found in or near wheat fields. The Bayesian phylogenetic analysis grouped the isolates into two major monophyletic clusters (I and II) with high Bayesian probabilities ($P = 0.99$). Cluster I contained isolates obtained from wheat as well as other *Poaceae* hosts ($P = 0.98$). Cluster II was divided into three host-associated clades (Clades 1, 2 and 3; $P > 0.75$). No morphological or cultural differences were observed among these species, but a distinctive pathogenicity spectrum was observed.

Micel *et al.* (2013) used 11 microsatellite loci to elucidate the population structure of the wheat blast pathogen (*Magnaporthe oryzae*) in wheat fields in central-western, southeastern and southern Brazil. No subdivision was found among the populations, consistent with high levels of gene flow across a large spatial scale. Although the clonal fraction was relatively high and the two mating type idiomorphs (MAT-1 & MAT-2) were not at similar frequencies. They also proposed that, populations of wheat blast pathogen exhibit a mixed reproductive system in which sexual reproduction is followed by the local dispersal of clones.

Srivastava (2014) evaluated 10 isolates of *Pyricularia oryzae* causing rice blast disease for their morphological, pathogenic, virulence and genetic characterization using RAPD marker. Isolates were classified into three groups based on pathogenicity viz. severely pathogenic, moderately pathogenic and mildly pathogenic and also four other groups i.e. PG-I, PG-II, PG-III, PG-IV based on cultural and morphological characteristics. The molecular polymorphism among isolates by means were analyzed by means of RAPD-PCR and the genetic coefficient matrix derived from the scores of the RAPD profile showed that minimum and maximum percent similarities among isolates were in the range of 80 to 35 % respectively.

Lima and Minella (2003) said that, the *P. oryzae* pathotype *Triticum* is considered the causal agent of wheat blast in South America and has also been associated with blast disease on barley, rye, triticale, and signal grass (*Urochloa* sp., ex *Brachiaria* sp.) in central-western and southern Brazil.

2.4. Management of *Magnaporthe oryzae* Pathotype *tritricum* causing blast by using plant extracts

Thi *et al.* (2016) evaluated that the fungicidal activity of extracts of *Houttuynia cordata* and *Artemisia indica* against rice blast caused by *M. oryzae* for new fungicide development. When spores of *M. oryzae* were suspended in an extract of *H. cordata* or *A. indica* and transferred onto glass slides, spore germination and appressorium formation of *M. oryzae* were significantly inhibited. Furthermore, blast lesion formation by *M. oryzae* was significantly inhibited in the presence of both extracts, compared to distilled water. Thus, extracts of *H. cordata* and *A. indica* might be the candidate control agents for plant protection against diseases such as rice blast.

Kumar *et al.* (2016) observed the effects of the bio control agents viz. *Trichoderma viride*, *T. harzianum*, *Pseudomonas fluorescens* and botanicals neem oil and neem oil + neem leaf extract (*Azadirachta indica*) against blast of rice (Pusa Basmati 1121). Carbendazim was used for standard check fungicides for comparison. The results concluded that the *P. fluorescens* inhibit the blast (21.99%) as compared to treated and untreated control (18.57% and 34.15 %, respectively) when *T. viride* is the best bio-control (24.53%) agent which was followed by *T. harzianum* (23.08%), neem oil (26.20%) and neem oil + neem leaf extract (24.15%) inhibit the blast respectively.

Jantasorn *et al.* (2016) 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 *P. oryzae*, *P. palmivora* and *R. solani* followed by *S. rolfsii* at 96.33 % when compared with water control. *X. lanceatum* fruit extract that logged excellent inhibitory activity against *P. oryzae*.

Shafaullah¹ (2016) experienced that garlic, ginger , chili, onion and eucalyptus have best control on *Pyricularia oryzae*. After 3rd week of Chili extract application, chili was proved best botanical pesticide against rice neck blast, whereas onion and garlic extract showed equal efficacy on eliminating neck blast. Eucalyptus was found least effect on leaf and neck blast.

Hubert *et al.* (2015) showed the effect of aqueous extracts of *Aloe vera*, *Allium sativum*, *Annona muricata*, *Azadirachta indica*, *Bidens pilosa*, *Camellia sinensis*, *Chrysanthemum coccineum*, processed *Coffee arabica*, *Datura stramonium*, *Nicotiana tabacum* and *Zingiber officinalis* for control of rice blast disease (*Pyricularia grisea*) *in-vitro* and *in-vivo*. 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*. Aqueous extract from *N. tabacum* at 10% concentration ranked third (80.35%) in inhibiting *P. grisea*. These were followed by extracts from 25% *A. vera* (79.45%) and 25% *C. coccineum* flower (78.83%). The results also indicate that, extracts from *A. indica*, *A. vera*, *A. sativum*, *C. arabica*, *D. stramonium*, *C. sinensis*, *Z. officinalis* and *N. tabacum* did not have any phytotoxic effect on seed germination, shoot height, root length, dry weight, seedling growth and seedling vigour index. These plant extracts can be used for rice seed treatment to manage rice blast disease.

Suriani (2015) proposed that 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. Results of this study showed that the crude extract of *P. caninum* exhibited a very strong inhibitory activity against *P. oryzae* with diameter of inhibition zone by 44 mm. Minimum inhibitory concentration (MIC) of this extract was 0.5% (w/v). Treatment with leaf extract of *P. caninum* significantly ($P < 0.05$) inhibited the fungal radial growth, spores formation, and biomass formation.

Manjappa (2015) evaluated that the Eupatorium (*Chromolaena odorata* L.) an obnoxious introduced 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 extent of inhibition of fungus was more with higher concentrations of extracts. 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. The incidence of neck blast was also less in eupatorium extract sprayed treatments.

Pandey(2015) observed that Aqueous extract of leaves of *Azadirachta indica*, *Emblica officinalis*, *Pongamia glabra* and *Acacia nilotca* were tested in-vitro at 0.2% and 0.5% concentration using poisoned food technique for antimicrobial activity against mycelial growth of *Magnaporthe oryzae* causing leaf blast and *Bipolaris oryzae* causing brown spot in rice. The leaf extracts were found significantly effective in reducing mycelial growth of the pathogens. The result reveals that *A.indica* leaf extract @ 0.5% was found most effective in minimizing the mycelial growth of both the pathogens 28.35 mm and 27.12 mm, closely followed by *P.glabra* leaf extract 29.57 and 30.10 mm in the same concentration, 96 hrs after incubation.

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.

Hajano *et al.* (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.

Sukanya(2011) observed that the essential oils and oleoresin from *Piper nigrum*, *Coriander sativum* and *Curcuma domestica* for their antifungal effect on *Pyricularia oryzae* in rice. Maximum inhibition of mycelial growth was observed in pepper oil which showed 5.9%, 5.5%, 4.4% and 7.0% of inhibition at 100ppm, 200ppm, 300ppm and control, respectively. Mycelial growth was completely inhibited at 500ppm and 1000ppm concentration. This indicated that pepper oil is most effective against *P. oryzae* pathogen.

Choi *et al.* (2004) used plant products as fungicides and found roots of *Chloranthus japonica* and stem of *Paulownia coreana* were effective in the management of rice blast they also noted that treatments with *P.guineense* and Carbendazim had comparable leaf blast suppression effects.

Miah *et al.* (1990) evaluated the efficacy of marigold (*Tagetes erecta*) extract for controlling *Pyricularia oryzae* and *Rhizoctonia solani*.

2.5. Management of other fungal pathogens with plant metabolites

Ahmed *et al.* (2013) reported that the efficacy of different plant extracts on reducing seed borne infection and increasing germination of collected rice sample. Five different plant extracts viz. garlic, allamanda, neem, chirata and biskatali with two dilutions (1:1 & 1:2) were tested for seed treatment. Garlic (1:10) was most effective in controlling seed-borne fungal flora of rice followed by neem (1:1) and chirata (1:1) extracts.

Neguefack *et al.* (2013) found that plant extracts and an essential oil were effective for controlling rice disease, tillering, number of panicles and yield increase.

Mansur *et al.* (2013) reported that five plant extracts viz. garlic, allamanda, neem, chirata and bishkatali with two dilutions (1:1 & 1:2) were tested for rice seed treatment. Garlic extract (1:1) dilution found best for three varieties which successfully reduced seed-borne infection and also increased seed germination up to 68.39% over control. Neem (1:1) and chitra (1:1) extracts also increased seed germination up to 66.09% and 67.81% respectively. Based on the present study, it may be concluded that among the five plant extracts with two dilutions (1:1 & 1:2), garlic (1:1) is most effective in controlling seed-borne fungal flora of rice followed by neem (1:1) and chirata (1:1).

Yeasmin *et al.* (2012) treated seeds of rice with Garlic (*Allium sativum*) clove extract at 1:0; 1:1; 1:2 dilution in water, Allamanda (*Allamanda cathartica*) leaf extract at 1:1; 1:2; 1:3 dilutions in water and Peovax-200 at 0.3% for controlling seed borne fungi where the seed samples of three rice varieties viz. Katharee, Gutee Aus and Kalizira were collected from farmer's storages of Bangladesh. All the treatments significantly reduced the seed borne fungi up to 100% over control, where Provax was found best and was statistically similar to garlic (1:1) extract against seed borne pathogen of rice.

Nisa *et al.* (2011) tested carbendazim, hexaconzole, bitertanol, myclobutalin, mancozeb, captan and zineb and extracts of *Allium sativum*, *Allium cepa* and *Mentha arvensis* for their effect on the inhibition of mycelial growth and spore germination of *Fusarium oxysporum*. maximum inhibition in mycelial growth was observed in the hexaconazole at 1000 ppm followed by other fungicides at the same concentration 'S'. it was followed by S/2, S/10 and S/100 concentrations of plant extracts as compared to control which showed least inhibition in spore germination.

Aye and Matsumoto (2011) reported the effect of some plant extracts on *Rhizoctonia spp.* and *Sclerotium hydrophilum*. All of the tested fungal growth were suppressed 100% by using clove extract, neem leaf, rosemary and pelargonium extracts were found to give the second best suppression against the tested fungi.

Roy *et al.* (2011) reported that five plant 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. Garlic tablet was effective in controlling seed-borne fungal infection; consequently the seed germination was high. The effect of allamanda tablet was similar to that of garlic tablet. Neem leaf extract was able to reduce seed-borne fungi but the other three extracts were not effective in controlling seed-borne infection. The performance of garlic tablet was similar to that of Vitavax-200. A significant increase in seedling vigor was also observed over untreated control after garlic treatment.

Singha *et al.* (2011) conducted an experiment with fourteen medicinal plants belonging to 13 families. Among all the extracts tested, PE, chloroform and methanol extracts of *Piper betel* L. And PE and chloroform extracts of *Allamanda cathartica* exhibited promising antifungal activity. Minimum inhibitory concentration (MIC) values of the above promising extracts were determined using broth dilution technique and observed that chloroform extract of *P. betel* L. exhibited the least MIC value ranged from 280 to 1130 mg/ml.

Kandhari *et al.* (2010) reported that essential oils, aroma compounds and plant extracts were effective against sheath blight of rice on a susceptible variety Pusa Basmati-1.

Masum *et al.* (2009) tested the effectiveness in controlling seed borne fungi associated with sorghum seeds with five seed treatment practices used viz. hot water treatment, garlic tablet, neem leaf extract, BAU-Biofungicide and Vitavax-200 which reduced significantly the total seed-borne fungal infections as well as the population of individual six target pathogenic fungi- *Agrotis tenuis*, *Bipolaris sorghicola*, *Botrytis cinerea*, *Crinum graminicola*, *Curvularia lunata*, *Fusarium moniliforme*. Vitavax-200 gave the best result in controlling seed-borne infection of all the individual target pathogenic fungi followed by garlic tablet, hot water treatment and neem leaf extract. BAU-Biofungicide gave the lowest control of total seed-borne fungal infections (61.6-62.3%) as well as seed-borne infection of all the individual target pathogenic fungi (<80.0%).

Sehajpal *et al.* (2009) evaluated the antifungal effect of 44 plant extracts and 8 plant oils against the pathogen *Rhizoctonia solani* by disc diffusion method. Among all the plant extracts, *A. sativum* exhibited strong fungi toxicity of inhibition 2.0 mm against the pathogen *Rhizoctonia solani*.

Kandhari (2007) reported the management of sheath blight of rice through fungicides and botanicals. All the fungicides and botanicals were found effective.

Hoassain *et al.* (2005) reported different plant parts of Biskatali, Vatpata, Garlic, Ghagra, Bitter gourd and Neem were tested against fungi associated with wheat seeds by blotter method. Out of 6 plant species, Neem extracts was turned up as superior among the selected extracts followed by Garlic, Biskatali and Vatpata.

Islam (2005) tested plant extracts such as Durba, Biskatali, Marigold, Mango, Pudina, and Allamanda were most effective in controlling seed borne fungi of rice.

Reddy *et al.* (2002) tested the efficacy of plant extracts and chemicals on the mycelial growth and sclerotial production of *Rhizoctonia solani* the pathogen of sheath blight of rice. Aspirin was highly effective (100%) inhibiting mycelial growth and sclerotial production followed by extracts of *Allium sativum* (61.81%) , *Azadirachta indica* (24.9%) and *Allium cepa* (19.3%). Neem formulations tested in glass house, neem fungicide (0.5%) and neem (*Azadirachta indica*) extracts (0.5%) were the best in controlling sheath blight disease of rice.

Hossain *et al.* (2001) tested plant extracts against *B. sorokiniana* and *R. solani*. The extracts of *Allium sativum* , *Cymbopogon nardus* and *Nigella saliva* showed better result in controlling the mycelia growth and sclerotial formations of *R. solani* and its pathogenecity to rice plants.

Naidu (1998) observed that the extract of deshi patabahar (*Codiaenum variegatum*) for antifungal activity and was found effective against *Alternaria alternata* and *Fusarium oxysporium* in vitro.

Ashrafuzzaman *et al.* (1990) reported that lemon grass oil and extract have antimycotic property against *Rhizoctonia solani*, the causal agent of sheath blight of rice. Lemon grass oil and extracts of *Citrus grandis*, *Mangifera indica* and *Anona squamosa* completely inhibited the growth of the fungus.

Rao *et al.* (1990) worked on management of seed-borne *Drechslera oryzae* of rice and found that maximum inhibition of *Drechslera oryzae* was obtained with extract of *Mentha pipertia* and *Piper nirgum* seed.

Alice and Rao (1986) worked on the management of *Drechslera oryzae* of rice with plant extracts and found that *Mentha piper* and *Allium sativum* extracts significantly reduced the infection of *D. oryzae* and observed that seeds treated with extracts produced seedlings of larger shoots and roots than those in the untreated check.

Tripathi *et al.* (1978) reported that the fungi toxic effect of higher plants *Lativsonia inermis* and found that the extracts of lawsonia leaves exhibited strong fungitoxicity. The antifungal factor was 2-hydroxy, 1, 4-napthoquinon (*Lawsonone*). The most effective dose against the test fungus *Helminthosporium oryzae* was 1000 ppm. Lawsonia was fungicidal with a wide fungitoxic spectrum and was non-phytoxic.

CHAPTER III

MATERIALS AND METHODS

The present research work was conducted to evaluate the effect of some plant extracts on controlling wheat blast caused by *M. oryzae* Pathotype *triticum* under laboratory and field Conditions.

3.1. Laboratory experiment

3.1.1. Experimental Site

The laboratory experiment was conducted in the laboratory of Department of Plant Pathology, Bangladesh Agricultural University and Plant Pathology Division, Bangladesh Institute of Nuclear Agriculture (BINA) Mymensingh.

3.1.2. Experimental period

The research work was performed during the period from August, 2016 - October, 2016.

3.1.3. Experimental design

The experiment was done following Completely Randomized Design (CRD) with three replications.

3.1.4. Treatments

Fifteen treatments including untreated control were explored in this experiment. The treatments were as follows:

T₁- Neem leaf extract (1:10)

T₂- Bishkatali leaf extract (1:10)

T₃- Nishinda leaf extract (1:10)

T₄- Alamonda leaf extract (1:10)

T₅- Acasia leaf extract (1:10)

T₆- Tulsi leaf extract (1:10)

T₇- Mehendi leaf extract (1:10)

T₈- Dhutara leaf extract (1:10)

T₉- Bishkachhu leaf extract (1:10)

T₁₀- Black cumin seed extract (1:10)

T₁₁- Garlic clove extract (1:10)

T₁₂- Mehogoni seed extract (1:10)

T₁₃- Provax (0.2%)

T₁₄- Nativo (0.2%)

T₀- Control

3.1.4.1. Specification of treatments

Specification of different treatments are given below (Table 1)-

Treatments	Scientific name/ Chemical name	Type	Plant parts used	Active ingredient
T ₁ : Neem	<i>Azadirachta indica</i>	Botanical	Leaf	Azadirachtin
T ₂ : Bishkatali	<i>Polygonum hydropiper</i>	Botanical	Leaf	Sesquiterpene dialdehydes polygodial, Warburganal
T ₃ : Nishinda	<i>Vitex negundu</i>	Botanical	Leaf	Hexadecanoic acid, Caryophyllene oxide.
T ₄ : Allamonda	<i>Allamanda cathartica</i>	Botanical	Leaf	Hexanoic acid, Octanoic acid
T ₅ : Acasia	<i>Acacia auriculiformis</i>	Botanical	Leaf	1,1-diphenyl-2- picrylhydrazyl (DPPH), Teracacidin
T ₆ : Tulsi	<i>Ocimum tenuiflorum</i>	Botanical	Leaf	Methyl chavicol, Linalool
T ₇ : Mehendi	<i>Lawsonia alba</i>	Botanical	Leaf	Anthraquinone
T ₈ : Datura	<i>Datura metel</i>	Botanical	Leaf	2beta-(3,4- dimethyl-2,5- dihydro-1H-

				pyrol-2-yl-1-methylethyl pentonate
T ₉ :Bishkachu	<i>Alocasia fornicata</i>	Botanical	Leaf	Alocasin
T ₁₀ :Black cumin	<i>Nigella sativa</i>	Botanical	Seed	Thymoquinone, Thymohydroquinone
T ₁₁ :Garlic	<i>Allium sativum</i>	Botanical	Clove	Allicin
T ₁₂ :Mehogoni	<i>Swietenia macrophylla</i>	Botanical	Seed	3B-acetoxymexicanolide, 6-acetylswietenine
T ₁₃ :Provax	-	Chemical	-	Provaxaltonin
T ₁₄ :Nativo	Methyl (2E)-2-methoxyimino-2-[2-[[[(E)-1-[3(trifluoromethyl)phenyl]ethylideneamino]oxymethyl]acetate+alfa-[2-(4-chlorophenyl)ethyl]-alfa-(1,1-dimethylethyl)	Chemical	-	Trifloxystrobin+ Tebuconazole
T ₀ :Control	without plant extract	Only PDA	-	-

3.1.4.2. Preparation of plant extracts

Plant extracts were prepared at 1:5 ratios of plant materials and water. About 20g of plant parts of each of them were chopped into small pieces. Water (100ml) was added to chopped sample and then blended. 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.



Figure 1: Different plant extracts

3.1.4.3. Preparation of Provax and Nativo solution (0.2%)

To prepare the 0.2% solution of Provax, 2 g fungicide was taken in an 1000 ml volumetric flask and then 1000 ml of distilled water was added up to the mark of flask. After that the content within the flask was stirred properly with the help of magnetic stirrer. By this way, 0.2% solution of Nativo was also prepared.

3.1.4.4. Preparation of PDA containing plant extracts

For this preparation 200g of clean peeled slice potato tubers were boiled in 500 ml of water. 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 for subsequent study.

3.1.4.5. Inoculation of PDA plate with *Magnaporthe oryzae* Pathotype *triticum* isolates

PDA plates supplemented with different plant extracts were inoculated with pure culture of *M. oryzae* Pathotype *triticum*. In brief, mycelial blocks were cut out of 10 days old fungal colony near the margin by means of sterilized cork borer of 5 mm diameter. These blocks were transferred to the center of the petri plates by means of a sterilized inoculating needle. For each of the treatments three replications were maintained. All these were done under aseptic condition inside a laminar air flow cabinet which was sterilized previously by spraying 70% Ethanol.

3.1.4.6. Measurement of mycelial growth rate

After 10 days, mycelial growth of *M. oryzae* Pathotype *triticum* were recorded by measuring the average of two diameters at right angles to one another. The same procedures were repeated three times and mean number of mycelial growth was considered.

Then the effect of plant extract was calculated as percent growth inhibition using the following formula reported by Satish et al. (2007); Dubey et al. (2009).

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

Where,

C= Mean mycelial growth (radial) of pathogen in control plate

T=Mean mycelial growth (radial) of pathogen in treatment

3.1.5. Data analysis

The data on different parameters were statistically analyzed by using analysis of variance (ANOVA) technique to find out the level of significance (Gomez and Gomez, 1984). The effect of the treatments was compared by Duncan's Multiple Range Test (DMRT). The collected data were analyzed using a statistically computer package MSTAT C.

3.2. Pot experiment

3.2.1. Experimental site

The experiment was conducted in the field laboratory of Plant Pathology Division, Bangladesh Institute of Nuclear Agriculture (BINA) and Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh.

3.2.2. Experimental period

The experiment was performed during the period from November, 2016 to March, 2017.

3.2.3. Crop and Variety

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

3.2.4. Preparation of experimental pot

Each of the plastic pot filled up with 10 kg soil which was silt-loamy in nature, low to medium organic matter, pH 5.1-5.6 belong to Old Brahmaputra Floodplain (AEZ-9). In total, 36 pots were prepared for 9 treatments including control and each of the treatment maintained with 4 replications.

3.2.5. Sowing of seed

Twenty (20) seeds in each pot were sown in 15 November, 2016. Thinning was done at 30 days after sowing (DAS) to maintain the optimum plant population.

3.2.6. Fertilizer application

The following fertilizers were applied in each pot as per recommended dose of the Fertilizer Recommendation Guide (BARC, 2012):

Fertilizer	Dose (Kg/h)	Dose(g/pot)
Urea	220	1
Triple Super Phosphate(TSP)	180	0.83
Muriate of Potash(MOP)	50	0.2
Gypsum	120	0.54
Boric acid	24	0.1

The one third of the Nitrogen and the total amount of TSP, MOP, Gypsum and Boric acid were applied in the soil during pot preparation. Rest of the urea was applied in two equal splits at 30 and 45 days after sowing (DAS).

3.2.7. Intercultural operation

Weeding was uniformly done in all the pots as per requirement. Irrigation was applied time to time when required.

3.2.8. Treatments

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 -

T₁- Garlic clove extract (1:10)

T₂- Black cumin seed extract (1:10)

T₃- Dhutara leaf extract (1:10)

T₄- Tulsi leaf extract (1:10)

T₅- Mehendi leaf extract (1:10)

T₆- Nativio (0.2%)

T₇- Control (Water)

3.2.9. Preparation of plant extracts

Different plant extracts were prepared at 1:10 ratio of plant materials and water. Sample of 100 g of plant parts of each of them were chopped into small pieces and about 400 ml of water was added to chopped sample and then blended. After that the material was filtered through the cotton cloth. Extract was taken in a conical flask. Then the volume was adjusted to 500ml by adding distilled water. The solution was auto calved for 30 minutes.

3.2.10. Preparation of fungicide 0.2% solution

To prepare the 0.2% solution of Nativio, 2g fungicide was taken in an 1000 ml volumetric flask and then 1000 ml of distilled water was added up to the mark of flask. After that the content within the flask was stirred properly with the help of magnetic stirrer. By this way, 0.2% solution of Nativio was prepared.

3.2.11. 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.

3.2.12. Inoculation of the growing plants

One day after application of the plant extracts and chemical, the treated plants were inoculated with mycelial suspension of *M. oryzae*. Ten days old culture of *M. oryzae* were used to prepare mycelial suspension with distilled water at 1:10 ratio (culture media: water). Then it was filtered through cheese cloth and 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.

3.2.13. Assessment of disease incidence and severity

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

$$\text{Disease incidence (\%)} = \frac{\text{No. of infected panicle}}{\text{Total no. of panicle}} \times 100$$

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. (Figure 2; Table 2):

Table 2: Disease severity scale (Maciel *et al.* 2013)

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

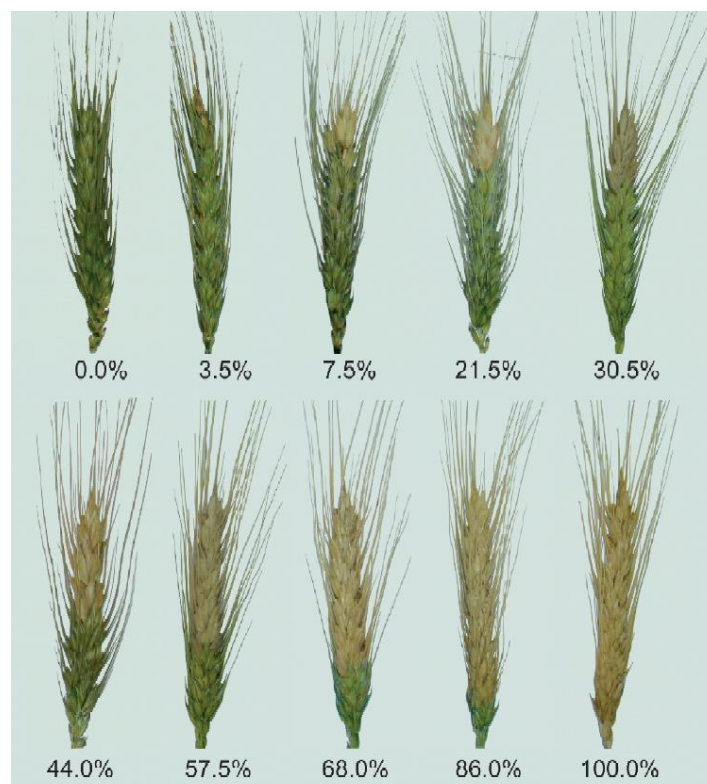


Figure 2: Diagrammatic scale for assessing blast severity caused by *Magnaporthe oryzae* Pathotype *triticum* on wheat spikes. The awns were not considered in the determination of severity on the spikes (Maciel *et al.* 2013)

3.2.14. Harvesting of crop

The crop was harvested on 28 February, 2017 at full ripening stage.

3.2.15. Data collection for the yield contributing parameters

Data on the following parameters were collected during the maturity stage of the plant.

- I. Plant height (cm)
- II. Ear length (cm)
- III. Number of ear/ pot
- IV. Number of healthy and diseased ear/ pot
- V. Number of spikelets /ear
- VI. Number of healthy and diseased spikelets/ear
- VII. Number of grains/ear
- VIII. Number of healthy and diseased grains/ear
- IX. Weight of grains/ear (gm)
- X. Weight of healthy and diseased grains/ear (gm)
- XI. 1000-grain weight (gm)
- XII. Grain yield/pot (gm)

3.2.16. Data analysis

The experiment was conducted in CRD with four replications. The data on different parameters were statistically analyzed using Analysis of Variance (ANOVA) technique to find out the level of significance (Gomez and Gomez, 1984). The treatment means were compared by Duncan's Multiple Range Test (DMRT) at 5% level of significance. The collected data were analyzed using statistical package MSTAT- C and Web Agri-Stat Package (WASP).

CHAPTER IV

RESULTS

4.1. Laboratory Experiment

4.1.1. Collection and Identification of wheat blast sample

Wheat blast infected plant samples especially panicles showing typical symptom were collected randomly from different wheat growing districts in Bangladesh in 2016. Five to ten different locations of each district were selected for sampling. From each location infected panicles of 10-15 plants were taken for the isolation of *Magnaporthe oryzae* Pathotype *triticum* (Figure 3).

Wheat blast pathogen *M. oryzae* usually attack panicle of wheat. So the most visible symptom of wheat blast in the field is bleaching of the spike. Partial or total spike sterility can occur depending on susceptibility of cultivar, timing, and point of infection. Infected awns show brown to whitish discoloration while infected glumes show elliptical lesions with reddish-brown to dark-gray margins.



Figure 3: Healthy (A) and wheat blast infected field (B) of wheat at Gangni, Meherpur, 2017

4.1.2. Isolation and identification of *Magnaporthe 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 was isolated from infected wheat spikes which were collected from farmer's field. Standard blotter method (ISTA, 1996) was used for the isolation of this *Magnaporthe oryzae* Pathotype *triticum* pathogen from infected spikes. In the 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 characters (**Figure 4**).

Fungal block was transferred to the centre of a fresh PDA plate using a sterilized block cutter. The plates were sealed with parafilm and all the isolates then grown under optimum conditions for 10 days. Sequential culturing from fungal stock was done for 4-6 times to get a pure culture which was used for inoculation (Figure 4). Inoculation was done in laminar air-flow cabinet. The incubation was done at 24°C with alternate 12/12 h UV light and darkness.

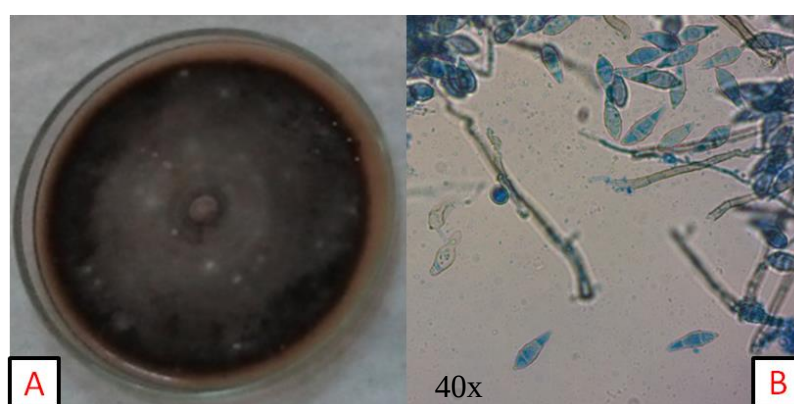


Figure 4: A pure culture of *Magnaporthe oryzae* Pathotype *triticum* on PDA (A) and different structures of *M. oryzae* (B)

4.1.3. Growth characteristics of *Magnaporthe oryzae* Pathotype *tritricum* on PDA media supplemented with different plant extracts

The growth characteristics like colour of substrate/media, color of colony and margin of colony of *M. oryzae* on PDA media supplemented with different plant extracts (1:10) were observed in this study. The color of the colony of *M. oryzae* having grey ash centre and black margin in case of PDA media. Whereas, PDA media supplemented with Neem leaf extract, 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 had an impact on the colony color of *M. oryzae* (Figure 6; Table 3). In addition, PDA media containing 0.2% fungicides such as Nativo and Provex showed no fungal colony after inoculation with *M. oryzae*.

On the other hand, margin of the pure colony of *M. oryzae* on PDA media and PDA supplemented with different plant extracts showed mostly regular form (**Table 3; Figure 5**).

Table 3: Colony characters of *M. oryzae* Pathotype *triticum* on PDA media supplemented with different plant extracts

Treatments	Colony characters		
	Substrate color/Media color	Colony color	Margin of colony
T ₀ =Potato dextrose agar (PDA)	Light brownish	Grey ash centre and black margin	Regular
T ₁ =Neem leaf extract + PDA	Green	White	Regular
T ₂ =Bishkatali leaf extract + PDA	Green	White	Regular
T ₃ =Nishinda leaf extract + PDA	Light green	White	Regular
T ₄ = Alamonda leaf extract + PDA	Light green	White	Regular
T ₅ = Acasia leaf extract + PDA	Dark green	White	Regular
T ₆ = Tulsi leaf extract + PDA	Red	Black	Regular
T ₇ = Mehendi leaf extract + PDA	Red	Black	Regular
T ₈ =Datura leaf extract + PDA	Dark Green	Black	Regular
T ₉ =Bishkachhu leaf extract + PDA	Lemon	Ashy center with white center	Regular
T ₁₀ =Black cumin seed extract + PDA	Black	Black	Regular
T ₁₁ =Garlic clove extract + PDA	Light Yellow	Black	Regular
T ₁₂ =Mehogoni seed extract + PDA	Orange	White	Regular
T ₁₃ =Provax (0.2%)	Blue	Black	Regular
T ₁₄ =Nativo (0.2%)	Creamy White	Black	Regular

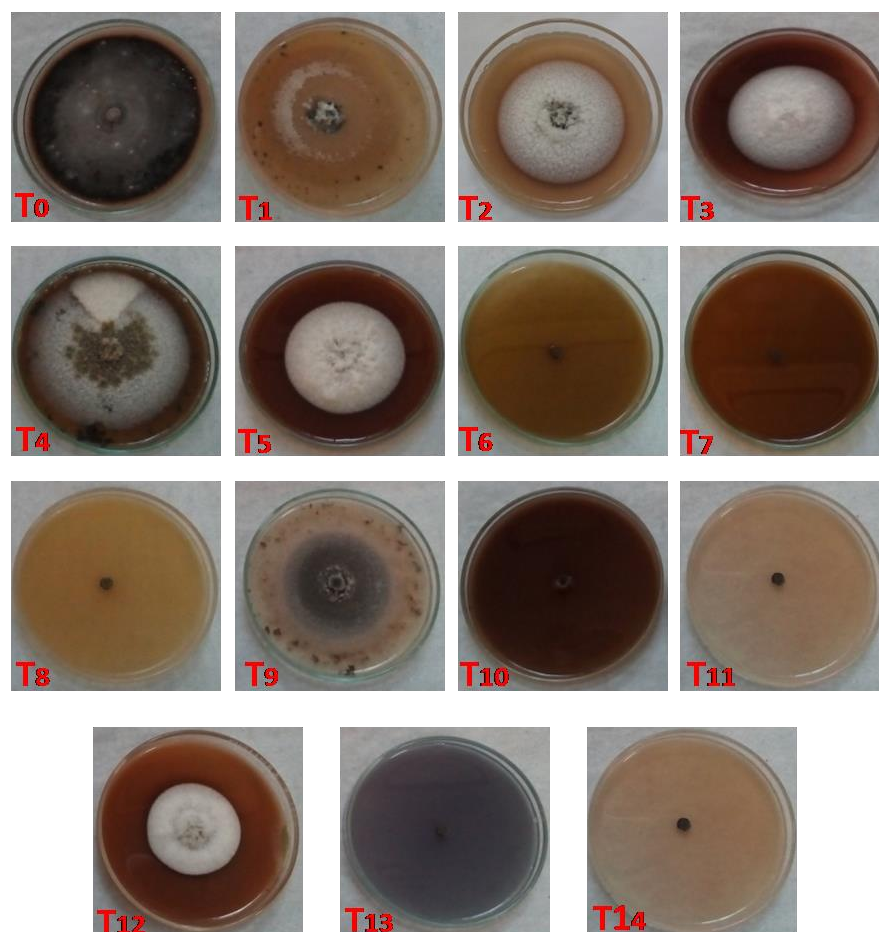


Figure 5: Status, colour and appearance of pure colonies of *M. oryzae* on PDA media supplemented with different plant extracts (Here, T₀=Potato dextrose agar, T₁= PDA+ Neem leaf extract, T₂= PDA+ Bishkatali leaf extract, T₃=PDA+ Nishinda leaf extract, T₄=PDA + Allamonda leaf extract, T₅=PDA+ Acasia leaf extract, T₆=PDA+ Tulsi leaf extract, T₇=PDA+ Mehendi leaf extract, T₈=PDA+ Datura leaf extract, T₉=PDA+ Bishkochu leaf T₁₀=PDA+ Black cumin seed extract, T₁₁= PDA+ Garlic clove extract, T₁₂= PDA+ Mehogoni seed extract, T₁₃= PDA + Provax 0.2%, T₁₄= PDA+ Nativo 0.2%)

4.1.4. Effect of different plant extracts on radial mycelial growth of *Magnaporthe oryzae* Pathotype *triticum*

Effect of some plant extracts were evaluated for controlling mycelial growth of *M. oryzae*. Mycelial growth of the pathogen was significantly controlled by some of the plant extracts viz. Garlic clove, Black cumin seeds, Tulsi leaves, Mehendi leaf and Datura leaf extract under *in-vitro* condition (**Table 4**). Mycelial growth in different treatments varied significantly where maximum mycelial growth was recorded under control (80 mm at 10 DAI) and minimum mycelial growth were recorded in the PDA

media supplemented with Tulsi, Mehendi, Datura and Garlic extracts (5 mm at 10 DAI) followed by Black cumin (8 mm at 10 DAI). Nativo and Provax were used as positive check and showed no mycelial growth on the PDA media (**Figure 5, Table 4**).

Table 4: *In-vitro* evaluation of different plant extracts on radial mycelial growth of *M. oryzae* Pathotype *triticum*

Treatments	Mycelial growth (mm) 5 DAI*	Mycelial growth (mm) 10 DAI*
T ₁ = Neem leaf extract (1:10)	31.5c	66.5c
T ₂ =Bishkatali leaf extract (1:10)	32bc	63.5d
T ₃ =Nishinda leaf extract (1:10)	20.5d	56.33e
T ₄ =Allamanda leaf extract (1:10)	34ab	74b
T ₅ =Acasia leaf extract (1:10)	21.75d	58.67e
T ₆ =Tulsi leaf extract (1:10)	5f	5i
T ₇ =Meheandi leaf extract (1:10)	5f	5i
T ₈ =Datura leaf extract (1:10)	5f	5i
T ₉ =Biskachu leaf extract (1:10)	22d	50.17f
T ₁₀ =Black cumin seed extract (1:10)	5f	8h
T ₁₁ =Garlic clove extract (1:10)	5f	5i
T ₁₂ =Mehogoni extract (1:10)	17.5e	43.5g
T ₁₃ =Provax(0.2%)	5f	5i
T ₁₄ =Nativo(0.2%)	5f	5i
T ₀ =Control	39.5a	80a
*LSD (0.05)	2.549	2.506

* DAI= Days after inoculation; Data represents the means of four replications.

*LSD (0.05): Least Significant Difference.

In a column, means followed by same letter(s) are statistically similar at 5% level by DMRT.

4.1.5. 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 *M. oryzae* Pathotype *triticum* 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 (**Figure 6, Table 5**) after 10 days of inoculation.

Table 5: Percent mycelial growth inhibition of *M. oryzae* Pathotype *triticum* in plant extracts supplemented with PDA media

Treatments	% Growth inhibition (5 DAI)	% Growth inhibition (10 DAI)
T ₁ = Neem leaf extract (1:10)	20.25	16.875
T ₂ =Bishkatali leaf extract (1:10)	18.987	20.625
T ₃ =Nishinda leaf extract (1:10)	48.1	29.587
T ₄ =Allamanda leaf extract (1:10)	13.92	7.5
T ₅ =Acasia leaf extract (1:10)	44.937	26.66
T ₆ =Tulsi leaf extract (1:10)	87.432	93.75
T ₇ =Meheandi leaf extract (1:10)	87.432	93.75
T ₈ =Datura leaf extract	87.432	93.75

(1:10)		
T ₉ =Biskachu leaf extract (1:10)	44.3	37.287
T ₁₀ =Black cumin seed extract (1:10)	87.432	90
T ₁₁ =Garlic clove extract (1:10)	87.432	93.75
T ₁₂ =Mehogoni extract (1:10)	55.696	45.625
T ₁₃ =Provax(0.2%)	87.432	93.75
T ₁₄ =Nativo(0.2%)	87.432	93.75
T ₀ =Control	-	-

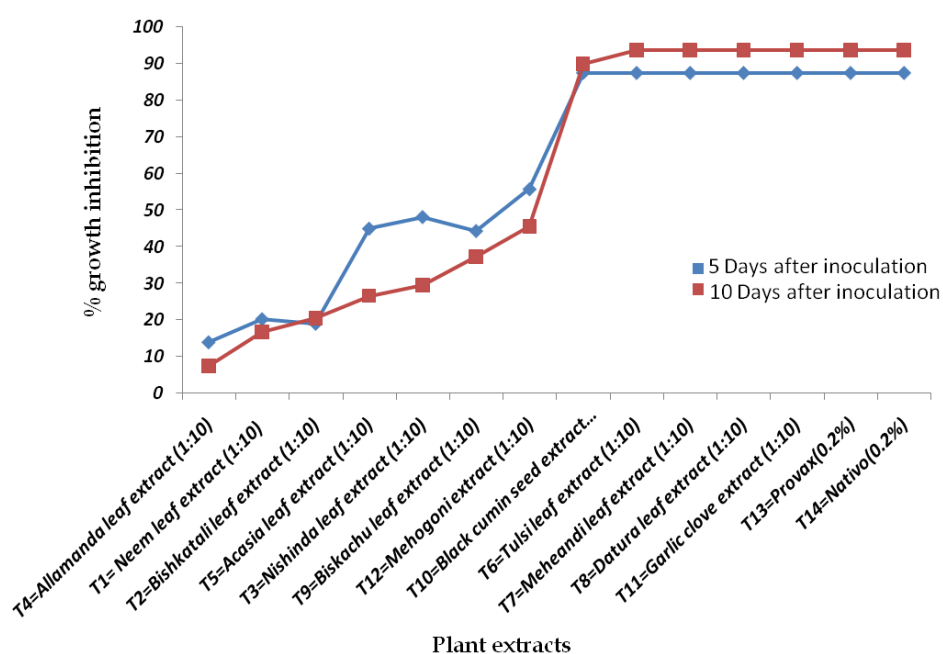


Figure 6: Percent (%) Growth inhibition of *Magnaporthe oryzae* Pathotype *triticum* on PDA plate containing plant extracts (1:10) separately over control

4.2. Pot Experiment

4.2.1. 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.

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%). On the other hand, chemical i.e. plants sprayed with Nativo showed very much similar results that showed by Garlic sprayed plants (**Table 6**).

Table 6: Effect of different plant extracts on the wheat blast incidence and severity

Treatments	Disease incidence (%)	Disease severity (%)
T ₁ =Garlic clove extract (1:10)	16.28	3.5
T ₂ =Black cumin seed extract (1:10)	34.78	7.5
T ₃ =Datura leaf extract (1:10)	51.92	44.0
T ₄ =Tulsi leaf extract (1:10)	62.5	57.5
T ₅ =Mehedi leaf extract (1:10)	66.0	68.0
T ₆ =Nativo (0.2%)	15.2	3.5
T ₀ =Control	98.11	100

4.2.2. Effect of plant extracts on the yield contributing parameter of wheat

4.2.2.1. Effect of foliar spray 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 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 (**Figure 7**).

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. Nativio sprayed plants showed very much similar results that showed by Garlic sprayed pot (Table 7)

Table 7: Efficacy of foliar spray of plant extracts on plant height, ear length, number of healthy and diseased ear / pot of wheat

Treatments	Plant height (cm)	ear length (cm)	no of ear/pot	no of healthy ear/pot	no of diseased ear/pot
T ₁ =Garlic clove extract (1:10)	86.92e	9.20a	13.25a	13.00a	0.25d

T ₂ =Black cumin seed extract (1:10)	87.45d	8.40b	13.25a	10.25b	3.00c
T ₃ =Datura leaf extract (1:10)	86.54e	8.40b	12.75b	5.50e	7.25a
T ₄ =Tulsi leaf extract (1:10)	88.32c	7.94c	12b	4.5f	7.50a
T ₅ =Mehedi leaf extract (1:10)	92.70a	9.06a	10.75d	9.00c	1.75cd
T ₆ =Nativo (0.2%)	87.60d	9.1a	12.80b	12.0	0.80d
T ₀ =Control	91.43b	8.30b	11.5c	7.5d	4.00b
LSD (0.05)	0.2028	0.3081	0.3132	0.2578	1.995

*LSD (0.05): Least Significant Difference.

In a column, means followed by same letter(s) are statistically similar at 5% level by DMRT.

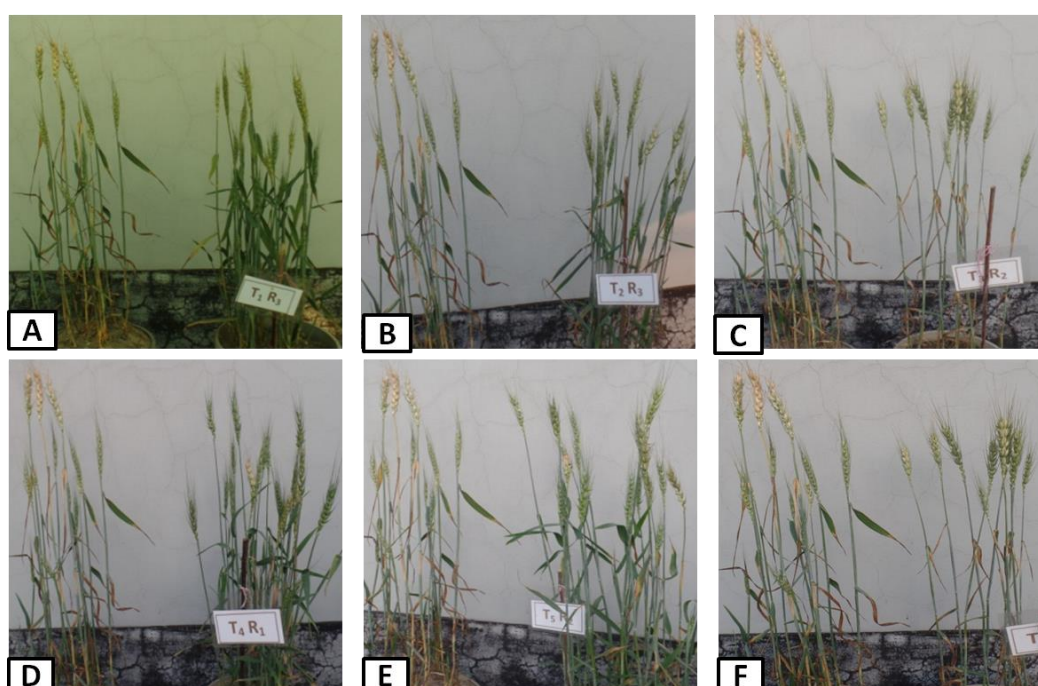


Figure 7: Effects of plant extracts on plant height, ear length, number of healthy and diseased ear / pot of wheat. Here, A= Garlic treated (right) and control (left), B= Black cumin treated (right) and control (left), C= Datura treated (right) and control (left), D=

Tulsi treated (right) and control (left) and E= Mehendi treated (right) and control (left), F= Nativo treated (right) and control (left).

4.2.2.2. Effect of foliar spray of plant extracts on number of healthy and diseased spikelets/ear, number of healthy and diseased grains/ear of wheat

Determine the effect of five selected plant extracts on number of total, healthy and diseased spikelets/ear and number of total, healthy and diseased grains /ear of wheat were studied in this experiment and the parameters showed significant differences under different treatments (**Table 8**).

Number of total spikelets/pot 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 plants 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 plants 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 plants showed very much similar results that showed by Garlic sprayed plants (**Table 8**).

Table 8: Effect of plant extracts on the number of total, healthy and diseased spikelets/ear and number of total, healthy and diseased grains/ear of wheat

Treatments	No of spikelets/ ear	No of healthy spikelets/ ear	No of diseased spikelets/ ear	No. of grains/ ear	No. of healthy grains/ ear	No. of diseased grains/ ear
T ₁ =Garlic clove extract (1:10)	34.20a	33.40a	0.80c	23.00a	21.80a	2.20e
T ₂ = Black cumin seed extract (1:10)	14.40f	12.40b	2.00c	13.40c	9.40c	4.00d
T ₃ = Datura leaf extract (1:10)	19.80d	7.00d	12.80b	14.40b	8.60d	5.80c
T ₄ = Tulsi leaf extract (1:10)	18.20e	6.00e	12.20b	11.40d	3.00e	8.40a
T ₅ = Mehendi leaf extract (1:10)	26.00c	11.20c	14.80b	10.60e	3.80e	6.80b
T ₆ = Natio (0.2%)	33.80a	33.30a	0.50c	22.40a	19.50b	2.90e
T ₀ = Control	29.40b	5.80f	23.60a	8.40f	0.00f	8.40a
*LSD (0.05)	0.2869	0.2869	2.546	0.2028	1.172	0.3328

*LSD (0.05): Least Significant Difference.

In a column, means followed by same letter(s) are statistically similar at 5% level by DMRT.

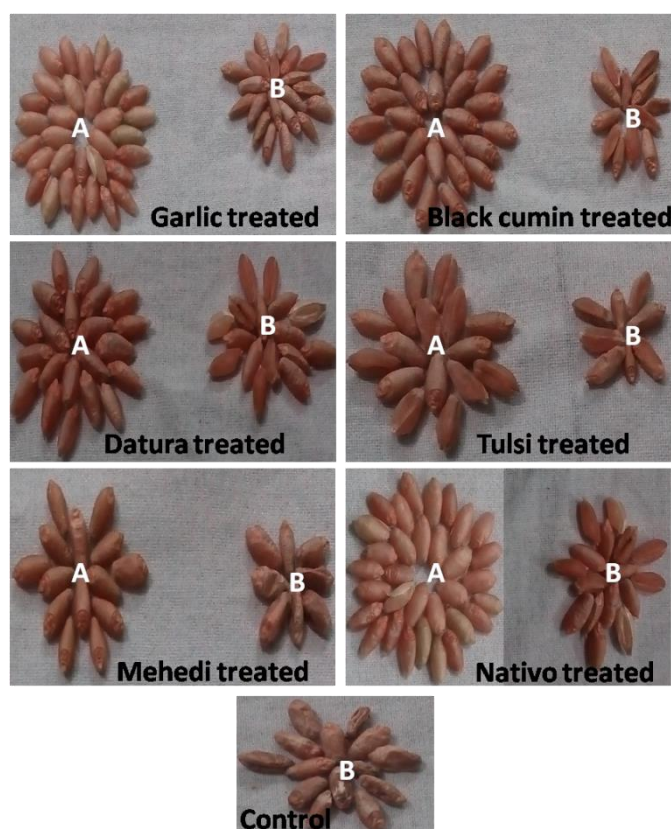


Figure 8: Efficacy of plant extracts on the number of healthy and infected grains/ear of wheat (Here, A= Healthy and B= Infected)

4.2.2.3. Effect of foliar spray of plant extracts on weight of healthy and diseased grains/ ear and weight of 1000 grain of wheat

To determine the effect of five selected plant extracts on weight of total, healthy and diseased grains (gm)/ ear and weight of 1000 seeds (gm) of wheat were evaluated and the parameters vary significant differences under different treatments (Table 9).

Weight of total, healthy and diseased grains/ear ranged from 4.72 gm to 1.80 gm, 4.20 gm to 0.00 gm and 0.52 to 2.00 gm for Garlic clove extract and control respectively. On the other hand the weight of 1000 seeds ranged from 56 to 37.4 gm 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 gm) followed by Nativo (4.6, 4.3 and 0.3 gm) where water sprayed control (1.80, 0.00 and 1.80 gm) showed the lowest value. On the other hand the weight of 1000 seeds Garlic clove extract (56 gm) sprayed plants showed better results

followed by Nativo (54gm) where water sprayed control (37.40 gm) showed the lowest value.

Table 9: Effects of five selected plant extracts on the weight of total, healthy and diseased grains/ ear and weight of 1000 grain of wheat

Treatments	weight of grains/ear (gm)	weight of healthy grains/ear (gm)	weight of diseased grains/ ear (gm)	weight of 1000 grain (gm)
T ₁ =Garlic clove extract (1:10)	4.72a	4.20a	0.52b	56b
T ₂ =Black cumin seed extract (1:10)	3.95b	2.60b	1.35ab	53.6a
T ₃ =Datura leaf extract (1:10)	3.15c	1.95bc	1.20ab	50a
T ₄ =Tulsi leaf extract (1:10)	2.59d	0.95cd	2.00a	46.5a
T ₅ =Mehedi leaf extract (1:10)	2.58d	0.73cd	1.85a	45a
T ₆ =Nativo(0.2%)	4.6a	4.3a	0.3b	54b
T ₀ =Control	1.80e	0d	1.80a	37.4a
*LSD (0.05)	0.9430	0.2578	0.5726	0.2808

*LSD (0.05): Least Significant Difference.

In a column, means followed by same letter(s) are statistically similar at 5% level by DMRT.

CHAPTER V

DISCUSSION

Wheat blast disease caused by *Magnaporthe oryzae* Pathotype *tritricum* is a new threat to wheat production which can causes yield losses up to 100% (Penga *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 (Islam *et al.*, 2016). So, management of wheat blast is very important for the sustainable production of wheat. Some chemical fungicides are used for controlling wheat blast, but they 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 *et al.*, 2011). 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 *M. oryzae*. The effect of plant extracts on radial mycelial growth (mm) of *M. oryzae* 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 of Jantasorn *et al.* (2016) 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 that logged excellent inhibitory activity against *P. oryzae*. Suriani (2015) proposed that 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. Pandey (2015) showed that aqueous leaf extract of *Azadirachta indica*, *Embllica 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.

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. Manjappa (2015) reported that the 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. 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 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. 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) followed by Black cumin seed extract (14.40, 12.40 and 2.00 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 black cumin seed extract. These findings are also corroborates the findings of earlier researchers. Hubert *et al.* (2015) evaluated 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*. 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. Choi *et al.* (2004) used plant products as fungicides and found roots of *Chloranthus japonica* and stem of *Paulownia coreana* were effective in the management of rice blast. They also noted that treatments with *P. guineense* and Carbendazim had comparable leaf blast suppression effects.

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,1999; Harris JC, *et al.* 2001 and Borlinghaus J, *et al.* 2014) and this compound might also inhibit the vegetative and reproductive growth of *P. oryzae* studied here. Moreover, garlic extract might also trigger the genes which are involved with the induced resistance in the host plants (Ghazanfar MU *et al.* 2011; Satya, V.K., *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 the control blast disease of wheat might be one of the 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.

CHAPTER VI

SUMMARY AND CONCLUSION

Wheat blast is caused by *Magnaporthe oryzae* Pathotype *tritici* 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.

The experiment was conducted in Plant Pathology Division, Bangladesh Institute of Nuclear Agriculture (BINA) and Department of Plant Pathology, Bangladesh Agricultural University, Mymensingh. The Efficacy of twelve different plant extracts (Neem, Bishkatali, Nishinda, Alamonda, Acasia, Tulsi, Mehendi, Datura, Bishkochu, Black cumin, Garlic and Mehogoni) were tested against *M. oryzae* in *in vitro* to select the best effective plant extracts(s) using the standard procedure of poison food technique for observing radial mycelial growth and percent growth inhibition on PDA media @ 1:10 concentration till 10 days post inoculation.

The result of the *in vitro* study clearly showed that the maximum inhibition (93.75%) of *M. oryzae* mycelial growth was obtained by Garlic, Datura, Tulsi and Mehendi followed by Black cumin extracts, Whereas Allamonda leaf extract was found least effective, as it inhibited only 7.5% of fungal mycelial growth on PDA plate. Based on *in vitro* experiment best five antifungal botanical extracts viz. Garlic, Datura, Tulsi, Mehendi and Black cumin were used to evaluate the disease incidence and severity and some yield contributing parameters in treated and control plants in the pot experiment. Minimum percentage of disease incidence and severity were observed in case of the plants treated with Garlic clove extract (16.28 and 3.5) and the Mehendi leaf extract treated plants showed highest percent of disease incidence and severity (66.0% and 68.0%). Plants sprayed with Garlic clove extracts also showed

best performance for yield contributing parameters like 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) followed by Black cumin , whereas Mehendi treated plants showed lowest value for all the yield contributing parameters among the plant extracts used in this experiment.

Based on the findings of the present study it may be concluded that Garlic cloves extract was the most effective under *in vitro* as it inhibited mycelial growth up to 93.75%. In additions, garlic clove extract may 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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