ECOFRIENDLY MANAGEMENT OF WHEAT BLAST DISEASE CAUSED BY *Magnaporthe oryzae triticum*

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CERTIFICATE

This is to certify that the thesis entitled **“ECOFRIENDLY MANAGEMENT OF WHEAT BLAST DISEASE CAUSED BY *MAGNAPORTHE ORYZAE TRITICUM*”** submitted to the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE in PLANT PATHOLOGY, embodies the result of a piece of bona-fide research work carried out by **ASHRAFUL AMIN MESHUK** bearing **Registration No. 13-05402** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that such help or source of information, as has been availed of during the course of this investigation has duly been acknowledged.

Dated: 17-01-2022
Dhaka, Bangladesh

Dr. F. M. Aminuzzaman
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DEDICATED TO

MY

BELOVED PARENTS

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ABSTRACT

The experiment was conducted in the experimental field, Central Farm, allotted for the students of Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207, during November 2020 to April 2021 to find out best ecofriendly management for blast disease of wheat. Three variety Prodip, BARI Gom-26 and BARI Gom-28 was tested to find out highest germination percentage and blast disease incidence (%). Among these varieties Prodip showed highest germination (93%) and blast disease incidence (30%). Seven treatments, *viz*. T_1= Control, T_2= Garlic clove extracts, T_3= Aloe vera leaf extracts, T_4= Black cumin seed extracts, T_5= Neem leaf extracts, T_6= Nativo 75 WG and T_7= Provax 200 WP, were used for blast disease management. The experiment was laid out in Randomized Complete Block Design (RCBD) design with three replications. Data were recorded on blast disease incidence (%), disease severity, growth and yield components of wheat. Experiment results concluded that among all treatments T_7 (Provax 200 WP) and T_5 (Neem leaf extract) performed better compared to other treatments for controlling blast disease of wheat. With T_7 (Provax 200 WP) treatment the lowest blast disease incidence (%) recorded was 7.86, 9.86 and 10.19, respectively, during the milking stage, soft dough stage, and hard dough stage of wheat. With T_5 (Neem leaf extract) treatment, a statistically equivalent reduction in blast disease incidence (%) was observed. During the milking stage, soft dough stage, and hard dough stage of wheat, the lowest blast disease severity was 1.03, 1.23 and 1.63, respectively, with T_7 (Provax 200 WP) treatment. A statistically similar reduction in blast disease severity was found with T_5 (Neem leaf extract). Therefore, it could be concluded that the treatment T_5 (Neem leaf extract) would be recommended as ecofriendly management for blast disease of wheat.

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ABBREVIATIONS AND ACRONYMS

FULL WORD	ABBREVIATION
Agricultural	Agril.
Agriculture	Agric.
American	Am.
And	&
And others	*et al.*
At the rate of	@
Bangladesh Agricultural University	BAU
Bangladesh Bureau of Statistics	BBS
Biology	Biol.
Continued	Cont'd
Centimeter	cm
Days after inoculation	DAI
Degree Celcius	^0c
Degree of freedom	df.
Gram	g
International	Intl.
Journal	J.
Mililitre	ml
Namely	*Viz.*
Negative logarithm of hydrogen ion concentration	$_P{}^H$
Number	No.
Pathology	Pathol.
Percentage	%
Research	*Res.*
Science	*Sci.*
That is	i.e.
Volume	Vol.

CHAPTER I

INTRODUCTION

Wheat (*Triticum spp.*) is a cereal grain originated from the Levant region of the Near East but now cultivated worldwide. It is estimated that approximately one-third of the world's people depend upon wheat for their nourishment (Hasan, 2017). Wheat forms the base of global food security, providing 20% of protein and calories of majority of the population in developing countries (Saharan *et al.* 2016). In 2020, world production of wheat was 758.3 million tons (FAO, 2020), making it the third most produced cereal after maize and rice. The human population is rapidly increasing and which needs a substantial increase in agricultural productivity worldwide. However, various biotic and abiotic stresses are major factors limiting crop productivity (Wani and Sah, 2014). To feed the world population, productivity must be increased by 70% for an additional 2.3 billion people by 2050 (Tilman *et al*., 2011).

Bangladesh is an agro-based country. Livelihood of 80% population of Bangladesh is directly or indirectly dependent on agriculture (Shamim, 2021). By considering annual production wheat is the second important cereal after rice in Bangladesh (BBS, 2015). The total area under wheat crop has been estimated 332274 ha, total production 1029354 ton and average yield 3.098 tha^{-1} (BBS, 2020). In Bangladesh, wheat provide 7% of the total output of food cereals. (BBS, 2015).

Increasing rate of emerging fungal diseases in crop plants is a serious threat to food and nutritional security of increasing population in the world (Fisher *et al.* 2012; Pennisi, 2010). Wheat diseases are responsible for 10–28% of yield losses worldwide (Figueroa *et al*., 2018; Savary *et al*., 2019). Foliar diseases of wheat caused by fungi are rusts, leaf rust, Septoria leaf blotch, spot blotch, tan spot, blast and powdery mildew (Simón *et al.* 2020). Among them one of the striking foliar diseases is the new emergence and re-emergence of blast disease which is caused by distinct pathotypes of a filamentous fungus *Magnaporthe oryzae* (Igarashi, 1986; Inoue *et al.* 2017). Presently, wheat blast disease has emerged as a threat for global wheat production (Cruz and Valent, 2017). It has been considered as a serious threat to 3 million ha of wheat cultivated area in South America since its first emergence in Paraná state in Brazil in 1985 (Igarashi *et al*. 1986; Goulart *et al.* 1992, 2007; Kohli *et al.* 2011). A 2009 outbreak in wheat cost Brazil one-third of that year’s crop (Shaharan *et al.* 2016). There are regions in South America where they don’t grow wheat because of the disease (Callaway, 2016). Wheat blast reached Itapúa and Alto Paraná Departments of Paraguay in 2002 (Viedma, 2005), and the province of Formosa in northeastern Argentina in 2007 (Cabrera and Gutiérrez 2007). In 2012, blast was detected in an experimental station within the Buenos Aires Province, potentially threatening important wheat production areas of Argentina (Perello *et al.* 2015). Wheat blast was spotted in Kentucky in 2011, however, vigorous surveillance helped to stop its spread in United States of America (Callaway, 2016). The production losses caused by *Pyricularia* blast can vary from very low to 100% (Goulart *et al.*1992). The disease can occur on all above ground parts (Saharan *et. al.* 2016). Highest loss occurs when fungal infection occurs at the base of the rachis restricting the development of grains and killing of spike (Kohli *et al*., 2011). First epidemic in Paraguay caused more than 70% losses (Viedma, 2005). Infection in low lands of Santa Cruz region of Bolivia in 1996 resulted in 80% yield reduction in wheat (Barea and Toledo, 1996).

The status of food security and food safety are alarming in Bangladesh due to crop losses by pests, diseases, emerging newly and resistant crop pathogens, and use of excessive synthetic pesticides (Callaway, 2013; Savary *et. al.* 2012; Islam *et al.* 2019). In 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 (Malaker *et al.* 2016). This first incidence of wheat blast affected approximately 15% of Bangladesh's total wheat area (Cruz and Valent, 2017). National yield rate has been declined in 2016 which is 1.78% lower than 2015 due to blast disease infestation (BBS, 2016). Government owned blast infected fields were burnt in Bangladesh (Callaway, 2016). Based on the molecular characteristics, wheat blast absolute from Bangladesh was found similar to that of Brazil (Malaker *et al*., 2016).

Plant diseases impact negatively on human well-being through agricultural and economic loss and also have consequences for biodiversity conservation (Ghalem, 2016). Wheat blast is a seed borne disease (Mahmud, 2019). Diagnosis of wheat blast disease in the field at heading stage is difficult because it produces similar symptoms as those associated with *Fusarium* head blight (FHB) (Pieck *et al*. 2017). First visible symptom on the leaf is water soaked and diamond-shaped lesions which turn into eye shaped gray lesions with disease progression. The eye-shaped lesions enlarge and coalesce with other lesions to kill the entire leaf. The most distinguishable symptom is observed in the head, which becomes partially or fully bleached (Islam *et al.* 2020). Infection at early stage of flowering results in sterility, and empty grains (Urashima *et al.* 2009). Infection at the grain filling stage results in small, shriveled, light, and discolored (pale) grains (Islam *et al.* 2016) and the affected grains are unfit for human consumption (Surovy *et al.* 2020). These grains are often discarded during the post-harvest process of threshing or winnowing (Urashima *et al.* 2009).

Seed treatment with Provax 200 WP (Carboxin 37.5 % + 37.5 % thiram) 3 gram per kg seed before sowing the seed may help to prevent this disease during seedling stage (BWMRI, 2018). Wheat blast pathogens attack both leaves and heads, the fungicide Nativo 75 WG 0.06 % (Tebuconazole 50 % + Trifloxystrobin 25 %) or Amistar Top 325 SC 0.1 % (Azoxistrobin 20 % + Difenoconazole 12.5 %) should be sprayed two times during the emergence of heading and after 15 days of first spraying in controlling the disease (Mahmud, 2019). Fungicide applications were only effective on flag leaves, but not on heads (Rocha *et al.* 2014). Fungicides are not effective in controlling wheat head blast if warm, rainy weather occurs during the heading stage (Urashima *et al.* 2009). The use of fungicide for the control of plant diseases is limited due to the possibility to the production of some pathogen populations resistant to fungicides and pathogen populations resistant to antimicrobial agents and the ability to the transfer of responsible resistant genes to human and animal pathogenic microbes. In addition, these chemical compounds can cause undesirable effects on environment due to their slow biodegradation and several serious side effects on mammalian health associated to toxic residues in agricultural products (Ghalem, 2016). Natural products which are safe for the environment and have low toxicity to living organisms are gaining interest as important sources for the development of fungicides, and these may serve as effective substitutes for synthetic fungicides (Martinez, 2012; Yoon, *et al.* 2013). Therefore, to increase food security and food safety and sustainable food production and to control wheat blast disease, we urgently need natural, environment friendly management options.

Objectives:

To evaluate the efficacy of selected botanical extracts in controlling wheat blast in the field.

CHAPTER II

RIVIEW OF LITERATURE

Wheat (*Triticum spp.*) is a cereal grain that lies at the heart of global food security, supplying 20% of the protein and calories consumed by the majority of people in poor nations (Saharan *et al.* 2016). Rusts, leaf rust, Septoria leaf blotch, spot blotch, tan spot, blast, and powdery mildew are fungi-caused foliar diseases of wheat (Simón *et al.* 2020). The fresh appearance and re-emergence of blast disease, which is caused by several pathotypes of the filamentous fungus *Magnaporthe oryzae*, is one of the most conspicuous foliar diseases (Igarashi, 1986; Inoue *et al.* 2017). Wheat blast disease is currently posing a danger to worldwide wheat output (Cruz and Valent, 2017). In Bangladesh, there is a scarcity of research on the management of wheat blast disease. However, some of the most recent and significant information on many areas of wheat blast disease control has been collated and provided below.

2.1. Importance of wheat

BBS (2014) reported that wheat provides 19% of the world's dietary energy supply, while rice supplies 20% and maize 5%. During 2012-13 and 2013-14, the world production has increased by 1% (from 472 MT s to 476 MT), trade by 8% (from 38 MT to 41 MT) and consumption by 3% (from 469 MT to 481 MT) (Commodity profile for rice - January 2015). BBS, (2014) also reported that wheat is the second major cereal crop (3% of total cereal consumption) after rice (93% of total cereal production) in Bangladesh.

Braun *et al.* (2010) reported that wheat (*Triticum aestivum*) is one of the most important cereal crops in the world. Wheat accounts for a fifth of humanity's food and is second only to rice as a source of calories in the diets of consumers in developing countries and is first as a source of protein.

FAOSTAT (2012) stated that wheat is an especially critical foodstuff for 1.2 billion people classified as 'wheat-dependent'; 2.5 billion are classified as 'wheat consuming' and live on, US$2 day21. There are also 30 million poor wheat producers and their families for whom wheat is the staple crop.

Islam *et al.* (2020) reported that wheat is the third largest cereal species produced in the world after corn and rice. It is the leading source of plant proteins in food for human consumption, as well as an important source of proteins for animal feed.

Rosegrant and Agcaoili (2010) reported that demand for wheat in the developing world is projected to increase 60 % by 2050.

Rosegrant *et al*. (1997) reported that the International Food Policy Research Institute projections indicate that world demand for wheat will rise from 552 million tons in 1993 to 775 million tons by 2020.

Saharan *et al.* (2016) reported that wheat forms the base of global food security, providing 20% of protein and calories of majority of the population in developing countries.

Shamim (2021) stated that wheat is the second staple food in Bangladesh after rice.

2.2. Significance of blast disease of wheat

CONAB (2017) reported that severe wheat blast epidemics in 1987 limited further expansion of the tropical wheat cropping areas into the Brazilian cerrado. As a result of wheat blast, wheat fields are now rare in the cerrado, dropping by 95% from 428,000 ha in 1987 to fewer than 20,000 ha in 2016.

Cruz and Valent (2017) reported that Blast has emerged as an explosive threat to wheat production that can cause up to 100% yield losses under the right environmental conditions.

Duveiller *et al.* (2016) reported that wheat blast has become a serious biotic constraint to wheat (*Triticum aestivum* L.) production in parts of the warmer wheat growing areas of the Southern Cone region of South America, causing yield losses of 10 to 100% in recent years.

Goulart and Paiva (1992) reported that blast infection at early stage of flowering results in sterility, and empty grains.

Goulart *et al.* (2007) reported that wheat blast is arguably the most yield-limiting wheat disease in Brazil, both in the traditional planting regions of southern Brazil as well as in the nontraditional region of the mid-west.

Hossain *et al*. (2019) reported that the emergence of the wheat blast in Bangladesh has generated severe threats to the food security of more than a billion people in South Asia.

Islam *et al.* (2016) reported that in the 2015–2016 wheat season in Bangladesh, wheat blast affected nearly 15,000 ha, or 3.5% of the total 436,817 ha of wheat cropland, and reduced wheat yield by 5–51% in the affected fields.

Mottaleb *et al.* (2018) warned that out of a total of 40.85 million ha of wheat cropland in Bangladesh, India, and Pakistan, nearly 7 million ha (17.1%) is vulnerable to wheat blast and there may be a loss of worth 132 million USD considering 5% blast-inflicted loss of wheat.

Peng *et al*. (2011) reported that wheat production in Bangladesh is under threat due to outbreak of devastating blast disease caused by *MoT.*

Shamim (2021) reported that wheat blast disease is wide spread in Bangladesh at this moment. The epidemic spread to an estimated 15,000 hectares, about 16% of the cultivated wheat area in Bangladesh, with yield losses reaching up to 100% which threatening food security of Bangladesh.

The production losses caused by *Pyricularia* blast can vary from very low to 100% (Goulart and Paiva, 1990; Goulart *et al.*1992). The disease can occur on all above ground parts. Highest loss occurs when fungal infection occurs at the base of the rachis restricting the development of grains and killing of spike (Kohli *et al*., 2011). First epidemic in Paraguay caused more than 70% losses (Viedma and Morel, 2002). Infection in low lands of Santa Cruz region of Bolivia in 1996 resulted in 80% yield reduction in wheat (Barea and Toledo, 1996). Government owned blast infected fields was burnt in Bangladesh (Callaway, 2016).

Wheat blast or *brusone do trigo* caused by fungus *Pyricularia* was first identified in the state of Parana, Brazil in 1985 (Igarashi *et al*., 1986) and caused a large-scale destruction of wheat. A 2009 outbreak in wheat cost Brazil one-third of that year’s crop. There are regions in South America where they don’t grow wheat because of the disease (Callaway, 2016).

2.3. Distribution of wheat blast disease

Wheat blast caused by the hemibiotrophic ascomycetous fungal pathogen *Pyricularia graministritici* (*Pygt*) (Castroagudın, *et al.* 2016) has been a major disease across central and southern Brazil since it was first described there in Parana State in 1985 (Igarashi, *et al.* 1986). Although it is highly likely that the disease was not widespread in the country before the first epidemic in 1985 (Igarashi, 1991), an earlier report from 1936 (Puttemans, 1936) indicated that the pathogen was already present in the Brazilian agroecosystem. After emerging in Parana, Brazil, the wheat blast pathogen spread to the neighboring states of Sao Paulo and Mato Grosso do Sul in 1986 (Goulart, *et al.* 1990) and Rio Grande do Sul in 1987 (Igarashi, 1991). The agriculture expansion to the warm cerrado areas of central western Brazil enabled the invasion of *Pygt* into the new wheat agroecosystems in Minas Gerais in 1990 (Lima, 2004), Goias in 1992 (Prabhu *et al*. 1992; Prabhu and Morais, 1993), and Brasilia in 1993 (Anjos *et al.* 1996). The pathogen spread to eastern Bolivia in 1996 (Barea and Toledo, 1996), eastern Paraguay in 2002 (Viedma and Morel, 2002; Viedma *et al.* 2010), and northern Argentina in 2007 (Alberione *et al.* 2008; Cabrera and Gutierres 2007; Perello *et al.* 2015). Starting from its origin in Parana, the pathogen spread into warmer regions 1,200 km north toward Brasılia; 1,700 km northwest toward Santa Cruz, Bolivia; and into cooler regions 1,200 km southwest toward the provinces of Chaco and Corrientes, Argentina (Ceresini *et al.* 2018). In 2012, blast was detected in an experimental station within the Buenos Aires Province, potentially threatening important wheat production areas of Argentina (Perello *et al*. 2015).

Malaker *et al.* (2016) reported that wheat blast was observed for the first time outside of South America during the 2015-16 cropping season in the districts of Kushtia, Meherpur, Chuadanga, Jhenaidah, Jessore, Barisal, Bhola and several other districts in the south of Bangladesh. This first incidence of wheat blast was significantly widespread accounting for approximately 15% of Bangladesh's total wheat area. They also reported that the air-dried spike samples collected from Bangladesh have been kept in cold storage and several infected spikes were sent to the USDA-ARS, FDWSRU laboratory in the United States for characterization of the pathogen. There, the presence of *M. oryzae* in the infected samples was confirmed based on morpho-biometrical analysis, and strains were preserved in the FDWSRU permanent wheat blast strain collection. Molecular analysis with *MoT*-specific markers and comparative genome analysis of isolates (BdBar16-1, GenBank accession no. LXON01000000; BdJes16-1, LXOO01000000; BdMeh16-1, LXOP01000000) confirmed that the wheat blast observed in Bangladesh is caused by *MoT* pathotype and has strong genetic identity to a strain from South America (B71, LXOQ01000000).

Ceresini *et al.* (2018) assumed that the wheat blast disease was introduced into Bangladesh through wheat grain trading from Brazil to Bangladesh.

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 (Farman *et al.* 2017; Malaker *et al.* 2016). An independent pathogenomics analysis confirmed that the Bangladeshi wheat blast fungus was most likely moved in from South America (Islam *et al.* 2016).

2.4 Causal organism of wheat blast disease

Castroagudın *et al.* (2016) reported that Wheat blast is caused by *Pyricularia graminis-tritici* (*Pygt*), a species genetically distinct from the *Pyricularia oryzae* species that causes rice blast.

Cruz and Valent (2017) reported that wheat blast is caused by a subpopulation within *M. oryzae*, the *M. oryzae Triticum* pathotype (MoT) that is distinct from sub-populations infecting rice (the *Oryza* pathotype, MoO); finger millet (the *Eleusine* pathotype); Italian or foxtail millet (the *Setaria* pathotype); and turf grasses (the *Lolium* pathotype, MoL); among others.

Geraldin *et al.* (2020), Islam *et al.* (2016) and Malaker *et al.* (2016) reported that wheat blast, a new devastating fungal disease caused by *Magnaporthe oryzae Tritichum* (MoT) has been observed in eight southwestern districts, viz., Meherpur, Chuadanga and so on. In February 2016, Bangladesh was reported as the first Asian country having an outbreak of worrisome wheat blast disease caused by a South American lineage of a hemibiotrophic filamentous fungus MoT pathotype.

Tanjina *et al.,* (2019) found that wheat spike blast was caused by *Magnaporthe oryzae* pathotype *triticum* (MoT) in south-western wheat growing regions of Bangladesh.

Goulart *et al*. (2007) reported that wheat blast, caused by *Magnaporthe grisea* (Cook) Sacc. (Anamorph: *Pyricularia grisea* (Hebert) Barr.).

Igarashi *et al.* (1986); Islam *et al.* (2016) reported that wheat blast which is caused by the ascomycetous fungus *Magnaporthe oryzae Triticum* (MoT) lineage (synonym *Pyricularia oryzae Triticum* lineage).

Initially the causal fungus of wheat blast was thought to be *Pyricularia oryzae* (telemorph *Magnoporthe oryzae*). Some authors named it as *Triticum* isolate of *Pyricularia oryzae* (Saharan *et al.* 2016). Sprague (1950) applied the name *P. oryzae* for rice isolates and *P. grisea* for other cereal grasses. Kohli *et al*. (2011) preferred name as *Pyricularia grisea* (Kooke) Sacc. [telemorph *Magnoporthe grisea* (Herbert) Barr]. Using a multilocus phylogenetic analysis, Couch and Kuhn (2002) described *M. oryzae* distinct from *M. grisea* and used former for isolates of rice, whereas later for perennial rye grass, wheat, millets and other grasses of agricultural importance. Recently Castroagudin *et al*. (2016) conducted phylogenetic assays using 10 housekeeping loci for 128 isolates of *P. oryzae* from sympatric populations of grasses growing in or near wheat fields. The analyses categorized isolates into three clades. Clade 1 comprised isolates associated with *P. oryzae* from rice. Clade 2 isolates belonged exclusively to wheat previously described as *P. oryzae* pathotype *Triticum*. Clade 3 contained isolates obtained from wheat as well as other Poaceae hosts. Clade 3 is distinct from *P. oryzae* and represents a new species *P. graminis tritici*. They claimed it to be the cause of wheat blast in Bangladesh. It appears to be a logical nomenclature of pathogen.

Inoue *et al.* (2017); Maekawa and Schulze-Lefert (2017) reporetd that the US wheat blast was likely caused by the 'host jump' *of M. oryzae Lolium* lineage from a native *Lolium* species.

Malaker *et al.* (2016) reported that Wheat blast or 'brusone,' caused by the ascomycetous fungus *Magnaporthe oryzae* B.C. Couch (syn. *Pyricularia oryzae* Cavara), was first identified in 1985 in Brazil.

The taxonomy of the *Pyricularia* species complex has long been disputed, oscillating between the names of the asexual (*Pyricularia*) or sexual (*Magnaporthe*) morphs to designate the genus (Ou 1985; Zhang *et al.* 2016). These taxonomy disagreements have resulted in naming the rice blast pathogen as four different species during the last 17 years (Tosa and Chuma, 2014): *Pyricularia grisea*, *Pyricularia oryzae*, *Magnaporthe grisea*, and *Magnaporthe oryzae.* A similar multiname species confusion exists for the wheat blast pathogen (Goulart *et al*. 1990; Igarashi,1991, Urashima *et al.* 2009; Urashima *et al.* 1993).

2.5. Disease symptoms and cycle

Cruz and Valent (2017) reported that the most visible symptom of wheat blast in the field is bleaching of the spike. An infection in the rachis or peduncle can block the translocation of photosynthates and kill the upper parts of the spik. As a consequence, 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. Lesions have grey centers during sporulation and white to tan centers after spore release (Igarashi *et al*. 1986; Igarashi, 1990). Grain fill is better when MoT infections occur later in the season; however, later infections may increase the chance of seed transmission of the pathogen with infected seeds (Igarashi, 1990).

Goulart *et al.* (2007) reported that the pathogen affects all aerial parts of the wheat plant, but the most characteristic symptom is observed in the spikes, which become bleached above dark lesions formed in the rachis, where the fungus sporulates abundantly. The bleached grain has no commercial value and yield losses of up to 74% can be incurred.

Islam *et al.* (2020) stated that first visible symptom on the leaf is water-soaked and diamond-shaped lesions which turn into eye-shaped gray lesions with disease progression. The eye-shaped lesions enlarge and coalesce with other lesions to kill the entire leaf. The most distinguishable symptom is observed in the head, which becomes partially or fully bleached. Complete or partial bleaching of the head above the point of infection with either no grain or shriveled grain with low test weight and poor nutrients is commonly observed. Multiple points of infection in the rachis can be observed and typically spread upward from its node. Whereas, infection at the grain filling stage results in small, shriveled, light, and discolored (pale) grains (Islam *et al.* 2016) and the affected grains are unfit for human consumption (Urashima *et al.* 2009; Surovy *et al*. 2020). Bleached heads have traces of gray, indicative of fungal sporulation at the point of infection (Islam *et al.* 2016). The white patchy symptoms at the reproductive stage are visible from a reasonable distance from the field.

Malaker *et al.*, (2016) reported that infected wheat plants in Bangladesh during the 2015-16 cropping season showed the typical wheat blast symptoms with the spike becoming partially or completely bleached with the blackening of the rachis in a short span of time. Examination of diseased plants showed the presence of elliptical, grayish to tan necrotic lesions with dark borders on the leaf often mixed with spot blotch disease lesions. Additionally, in some fields, blackening of lower nodes was observed. Grains from blast-infected heads were small, shriveled, deformed, and had low test-weight leading to serious yield losses.

Pieck *et al.* (2017) reported that diagnosis of wheat blast disease in the field at heading stage is difficult because it produces similar symptoms as those associated with Fusarium head blight (FHB).

Saharan *et al.* (2016) reported that wheat blast is mainly a disease of spike, however, it can produce lesions on all above ground parts under hot and humid conditions. Depending on the point of infection on rachis, it can cause breaking or drying of spike. Head infection can be easily confused with *Fusarium* head blight. Head infection can occur on the glumes, awns and rachis. Infected glumes support elliptical lesions with reddish brown to dark grey margins and white to light brown center. Blackening of the rachis, a lower node, shriveling of grains, low test weight has also been observed (Malaker *et al*., 2016). The pathogen is known to produce non host specific toxin pyricularin (Agrios 2005). Depending on the place of infection on the head, drying of partial or full ear head takes place. Symptoms on heads can vary from elliptic lesions and bleached centers, spike bleaching, sterility and empty grains depending on time of infection. Since infection on spike blocks the translocation of photosynthates and nutrients to spike, therefore, results in partial or total spike sterility. Grain fills can occur in case of late infections but it may lead to seed borne inoculum to the next crop. On leaves lesions vary in shape and size depending on the stage of plants. As plants grow older, lesions are less frequent. Lesions with white centre and reddish-brown margin on upper side, dark grey on the underside of the leaf can be observed on both young and old infected leaves. Infection on seedlings can be very damaging under high temperature and humidity and can lead to total plant death (Igarashi, 1990).

The infection cycle of the *Magnaporthe* fungus starts when the three-celled conidia attach to the plant surface. After that, conidia germinate to form polarized germ tubes from either one or both ends after 6 h of inoculation. Recognition of environmental cues such as surface hydrophobicity and toughness induces swelling at the tip of the germ tube, which then differentiates into the specialized infection structure called appressorium (Hamer *et al.* 1988; Tufan *et al.* 2009). The appressorium generates enormous turgor pressure by accumulating high concentration of compatible solute such as glycerol with the help of melanized cell wall. This pressure is converted into mechanical force, which powers the penetration peg to penetrate the leaf epidermis or stalk cuticle followed by further invasive hyphal expansion to colonize plant tissues (Kankanala *et al.* 2007; Tufan *et al.* 2009; Wilson and Talbot, 2009). Infection hyphae have cap-like structures known as the biotrophic interfacial complex involved in releasing effectors into the host plant cells (Kankanala *et al.* 2007; Mosquera *et al.* 2009; Giraldo *et al*. 2013). The MoO secrets some effector proteins such as Avr-Pita, Avr-Pii, Avr-Piz-t, Pwl1, ACE1, Bas1– 4, Slp1 and Mc69 that suppress the host immunity through binding chitin oligosaccharides in the apoplast and by targeting multiple components of the host immunity (Mentlak *et al*. 2012; Liu, *et al*. 2013; Chen *et al.* 2014). It also secrets antibiotic biosynthesis monooxygenase and the mycotoxin tenuazonic acid, which helps the fungus to colonize in plant tissue for successful.

Biotrophic growth of the fungus (Kankanala *et al*. 2007; Patkar *et al.* 2015; Yan and Talbot, 2016). The MoO also produces and secretes a diverse array of phytotoxic secondary metabolites to collapse host plants for successful infection (Nukina, 1999). However, precise underlying molecular cross-talks between wheat plant and MoT fungus are poorly understood. Disease lesions usually become apparent after 72–96 h of infection by the conidia (El Refaei, 1977; Islam *et al.* 2016). On the other hand, mycelial mass and spore production are observed after 120 h of inoculation on plant leaf surface (Ceresini *et al.* 2018).

2.6. Morphological characteristics of *Magnaporthe oryzae*

Malaker *et al.*, (2016) reported that grayish mycelium of the fungus taken from the infection point on the rachis of several independent spikes was observed. Incubation of several infected spikes/leaves in a 3-layered moist blotter at room temperature less than 12 h light/darkness cycle for 5 days led to the production of pyriform conidia.

Subramanian (1968) stated that typical pyriform (pear-shaped) and 2-septate hyaline conidia were in agreement with the identification of the fungus as *Magnoporthe oryzae*.

Ashrafi *et al*., (2021) stated that *Magnaporthe oryzae triticum* (MoT) isolates showed comparatively higher mean mycelial growth rate on Oat Meal Agar (15.43 mm per day) media followed by Potato Dextrose Agar (14.34 mm per day) at 4 DAI. Minimum mean mycelial growth rate was recorded on Potato sucrose Agar (14.02 mm per day).

Wheat blast, or ‘brusone’, is caused by the haploid, filamentous, ascomyceteous fungus *Magnaporthe oryzae* (Catt.) B.C. Couch 2002 (synonym to *Pyricularia oryzae Cavara* 1892) (Couch and Kohn 2002; Zhang *et al*. 2016).

2.7. Evaluation of eco-friendly management against *Magnaporthe oryzae*

Plant extracts, essential oils, gums, resins etc. have been shown to exert biological activity against plant fungal pathogens *in vitro* and *in vivo* and can be used as bio-fungicidal products (Fawzi *et al.*, 2009; Jalili *et al.*, 2010; Romanazzi *et al.*, 2012). These products are generally assumed to be more acceptable and less hazardous for the ecosystems and could be used as alternative remedies for treatment of plant diseases (Chuang *et al.*, 2007).

2.7.1. Use of *Aloe vera* as bio-fungicide

Agarry *et al*. (2005) investigate the comparative antimicrobial activities of *Aloe vera* gel and leaf. They tested *Aloe vera* gel and leaf against *Staphylococcus aureus, Pseudomonas aeruginosa*, *Trichophyton mentagraphytes*, *T. schoeleinii*, *Microsporium canis* and *Candida albicans*. Investigation result concluded that both the gel and the leaf inhibited the growth of *S. aureus* (18.0 and 4.0 mm, respectively).

They also reported that the gel inhibited the growth of *T. mentagrophytes* (20.0 mm), while the leaf possesses inhibitory effects on both *P. aeruginosa* and *C. albicans*.

Agbowuro *et al.* (2020) conducted a research work to evaluate different aqueous plant extracts against rice blast disease fungus (*Magnoporthe oryzae*). They used 5 aqueous plant extracts at different concentration (25, 50, and 100%) against rice blast disease (*Magnaporthe oryzae*) in-vitro and in-vivo. Result showed that *Aloe vera* plant extracts at 100% concentration inhibit 56.37% mycelial growth and almost 40% disease severity of *Magnaporthe oryzae*.

Barkai-Golan (2001) reported that *Alo vera* extract have antifungal activity against four common postharvest pathogens: *Penicillium digitatum*, *P. expansum*, *Botrytis cinerea* and *Alternaria alternata*.

Harlapur *et. al.* (2007) evaluated thirteen botanicals, eight bio-agents and twenty-three fungicides *in vitro* against turcicum leaf blight of maize caused by *Exserohilum turcicum* (Pass.) Leonard and Suggs. They concluded that *Aloe vera* @ 10 per cent concentration inhibit growth 53.50% of *Exserohilum turcicum* (Pass.) Leonard and Suggs.

Hubert *et al.* (2015) conducted an experiment to find out the efficacy 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*. Experiment results also showed that *Aloe vera* at 25% (v/v) had 79.45% inhibitory effect against *P. grisea*. They also reported 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 vigor index.

Khatun (2018) reported that *Aloe vera* leaf extracts @ (1:1 w/v) concentration was one of the most effective botanicals to reduce mycelial growth of *M. oryzae triticum* under *in-vitro* condition.

Nazifa *et al.* (2021) investigated *In Vitro* efficacy of botanicals against rice rlast pathogen *Magnaporthe oryzae oryzae*. Eight botanicals extracted both in water and ethanol were tested against *Magnaporthe oryzae oryzae* in vitro condition. Investigation results concluded that among all eight botanicals *Aloe Vera* 1:4 (w/v) was the least effective botanicals with 7.86% inhibition of mycelia radial growth at 7 DAI.

Pawar *et al.* (2005) completed a study to find out the antibacterial activity of *Aloe vera* leaf gel extracts against *Staphylococcus aureus*. Study result concluded that 50% *Aloe vera* leaf gel concentration completely inhibit the growth of *S. aureus*.

Saks and Barkai-Golan (1995) tested the antifungal activity of *Aloe vera* gel at 1–10^5 µl l^{-1} on four common postharvest fruit pathogens: *Penicillium digitatum, P. expansum, Botrytis cinerea*, and *Alternaria alternata*. They reported that the natural *Alo vera* gel suppressed both germination and mycelial growth of *P. digitatum* and *A. alternata*. They also reported that spore survival of *P. digitatum*, *A. alternata*, and *B. cinerea* was reduced by 15–20% at 1 µl l^{-1}, but the gel was similarly effective against *P. expansum* only when the concentration exceeded 10^3 µl l^{-1}.

Uda *et al.* (2018) evaluated antimicrobial activity of plant extracts from *Aloe vera, Citrus hystrix*, Sabah snake grass and *Zingiber officinale* against *Pyricularia oryzae* that causes rice blast disease in paddy plants. From the results they reported that Sabah snake grass extraction has the highest inhibition value at concentration 30 mg/ml with 12 mm of the inhibition zone formed, followed by *Aloe vera* with 8.67 mm, *Zingiber officinale* with 8.33 mm and *Citrus hystrix* extraction with the diameter of the inhibition zone of 7.33 mm.

2.7.2. Use of Neem leaf extract as bio-fungicide

Achimu and Schlosser (1992) reported that raw neem seed extract had high antifungal properties against downy mildew (*Plasmopara viticola*) of grape vine.

Agbowuro *et al.* (2020) conducted a research work to evaluate 5 aqueous plant extracts in-vitro and in-vivo against rice blast disease fungus (*Magnoporthe oryzae*) at different concentration (25, 50, and 100%). They reporetd that neem plant extracts at 100% concentration inhibit 69.54% and 50% concentration inhibit 57.00% mycelial growth of *Magnaporthe oryzae*.

Ahmed *et al.* (2019) conducted a study to investigate the effect of some eco-friendly materials like waste agriculture extracts in order to reduce uses of chemical fungicides and to maintain sustainable development. Study result concluded that maximum inhibition of *Puccinia triticina* spore germination reached to 100% by using neem cake and rice straw extracts followed with orange peels extract.

Amadioha (2000) conducted an experiment to controlling rice blast in vitro and in vivo with extracts of *Azadirachta indica*. Experiment results concluded that oil extract of seeds of *Azadirachta indica* (neem) significantly reduced the in vitro radial growth of *Pyricularia oryzae* and the development and spread of blast disease in rice plants in the greenhouse. He also reported that *Azadirachta indica* appears to have the potential to be used for managing rice blast in the field.

Anamika and Simon (2011) evaluated the efficacy of plant extracts at 5% and 10% concentration on *Alternaria alternata* causing dry rot of *Aloe vera*. They reported that neem leaf extract showed 58.6% inhibition of radial growth of *Alternaria alternata*.

Butt *et al.* (2016) reported that at different concentrations (1%, 2%, 3%, 4% and 5%) *Azadirachta indica* leaf extract effective against *S. rolfsii* under *in vitro* condition.

Hajano *et al.* (2012) conducted an in-vitro evaluation of fungicides, plant extracts and bio-control agents against rice blast pathogen *magnaporthe oryzae* couch. They reported that neem extract @ 4ml/15 ml medium completely reduced growth of P. *oryzae*.

Harish *et. al.* (2007) tested fifty plant extracts, four oil cakes and eight antagonistic organisms against *Bipolaris oryzae* (*Cochliobolus miyabeanus*), the causal agent of brown spot disease of rice. They reported that neem cake extract showed the maximum inhibition percent to mycelial growth (80.18%) and spore germination (81.13%) of the pathogen. They also reported that two rounds of spraying of rice plants with neem cake extractin the field at initial appearance of disease and 15 days later reduced the 70% incidence of brown spot and increased the yield by 23%.

Harlapur *et. al.* (2007) evaluated thirteen botanicals, eight bio-agents and twenty-three fungicides *in vitro* against turcicum leaf blight of maize caused by *Exserohilum turcicum* (Pass.) Leonard and Suggs. They concluded that neem seed kernel extract @

five per cent concentration caused maximum inhibition of growth (56.64%) of *Exserohilum turcicum* (Pass.) Leonard and Suggs.

Hossain and Schlosser (1993) investigated the effect of neem seed extracts and neem oil cake against *Bipolaris sorokiniana*. They reported that *in vitro* condition neem seed extracts inhibited the growth of the fungus and reduced the pathogenicity in wheat leaves.

Hossain *et al*. (2005) reported that extract of different plant viz. bishkatali, vatpata, garlic, gagra, bitter gourd and neem were effective against fungi associated with wheat seed. Out of six plant species, neem extract was turned up as superior among the selected extracts followed by garlic, bishkatali and vatapta.

Hubert *et al.* (2015) conducted an experiment to find out the efficacy of aqueous extracts for control of rice blast disease (*Pyricularia grisea*) in-vitro and in-vivo. They concluded that *Azadirachta indica* at 25% (v/v) concentration inhibited 77.52% mycelial growth of *Pyricularia grisea*.

Kamalakannan *et al.*, (2001) studied the efficacy of different plant extracts against blast disease under pot culture conditions and reported that, pre and post inoculation spray of *Prosophis julifera, Zizyphus jujuba* and *Azadirachta indica* exhibited greater reduction in disease incidence.

Keta *et al.* (2019) carried out a study to determine effect of Neem (*Azadirachta indica* A. Juss) leaf extract in different concentrations of 5, 10 and 15ml/L on the growth of *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus nidulans* and *Aspergillus fumigatus* isolated from foliar diseases of rice. Effect of the extracts was determined by measuring radial growth (mm) of the isolated fungi on potato dextrose agar in petri-dishes. They reported that the ethanolic extract was more effective than aqueous extract in all case and the effectiveness of the extracts was dependent on the concentration used.

Meena and Muthusamy (1999) found that application of neem cake (150 kg/ha) in combination with palmarosa oil (0.1%) caused 73.6 per cent reduction in sheath blight disease incidence over control.

Mossini and Kemmelmeier (2008) investigated the efficacy of different concentrations of aqueous neem leaf extract (3.12 to 50 mg/mL) on growth and citrinin production in three isolates of *Penicillium citrinum* under laboratory conditions. Results concluded that mycotoxin production by the isolates was suppressed, depending on the concentration of the plant extract added to culture media at the time of spore inoculation, but failed to inhibit fungi mycelia growth.

Mossini *et al.* (2009) conducted an in vitro trial to evaluate the effect of *Azadirachta indica* (neem) extracts on mycelial growth, sporulation, morphology and ochratoxin A production by *P. verrucosum* and *P. brevicompactum*. They reported that oil extracts exhibited significant ($p \leq 0.05$) reduction of growth and sporulation of the fungi.

Nahak and Sahu (2014) investigate the bio efficacy of leaf extracts of neem (*Azadirachta indica* A. Juss) on growth parameters, wilt and leafspot diseases of brinjal. They reported that neem extracts as most effective agent in controlling leaf spot (82.33%) and wilt (41.34%) in comparison to control under field conditions.

Nashwa and Abo-Elyousr (2012) evaluated six plant extracts against the early blight disease of tomato plants under greenhouse and field conditions. They reported that during in vitro condition 5% concentration of *Azadirachta indica* caused 43.3% reduction of mycelial growth of *Alternaria solani*.

Nazifa *et al.* (2021) investigated *In Vitro* efficacy of botanicals against rice blast pathogen *Magnaporthe oryzae oryza*. Eight botanicals extracted both in water and ethanol was tested against *Magnaporthe oryzae oryzae* in vitro condition. They reported that ethanol extracts of neem (1:4 w/v) inhibits 92.62% mycelia growth at 14 DAI.

Pandey (2015) studied aqueous leaf extract of *Azadirachta indica, Emblica officinalis, Pongamia glabra* and *Acacia nilotca* inhibit the mycelial growth of *Magnaporthe oryzae triticum* causing leaf blast and *Bipolaris oryzae* causing brown spot in rice under laboratory condition.

Ramos *et al.* (2007) conducted and experiment to identify alternative, environmentally safe and cost-efficient methods for the control of withces' broom and pod rot of cocoa. They reported that 5% growth reduction of mycelia of P*hytophthora* on neem leaf extract media; whereas growth of mycelia of *Crinipellis* was not affected, even at the highest concentration of neem leaf extracts used (35%). They also reported that 20–35% concentration neem leaf extracts reduced germination of *Crinipellis* spore almost completely.

Regmi *et al.* (2014) evaluated leaf extracts of six plants against the fungus *Alternaria alternata* causing leaf spot disease of Aloevera and reported that neem (*Azadirachata indica*) leaf extracts inhibited 51.9% mycelia growth of the fungus *Alternaria alternata*.

Rijal and Devkota (2020) stated that neem extract 4ml/15ml, *Coffee arabica* @ 25%, *Nicotiana tabacum* @ 10% are effective but garlic extract @ higher doses and neem extract @ 4ml/15 ml are best for reduction of blast incidence.

Shabana *et al.* (2016) investigated the efficacy of eight plant extracts (garlic, clove, garden quinine, Brazilian pepper, anthi mandhaari, black cumin, white cedar and neem) in controlling leaf rust disease of wheat in vitro and in vivo. They reported that 93% Neem extract inhibited 98.99% of spore germination and 36.82% reduction in the amount of pustules/leaf.

Siddique *et al.* (2018) evaluated the efficacy of plant extracts against foot and root rot disease of eggplant caused by *Sclerotium rolfsii* and reported that the effect of neem extract on reduction of disease incidence and disease severity reduction over control on eggplant was recorded 60% and 58.64%.

Siresha and Venkateswarlu (2013) evaluated five plant parts extract namely Neem seed kernel extract, Neem oil, *Asafoetida* spp. and *Pongamia* spp. extracts and Panchagavya for their efficacy against blast of rice in in vitro conditions. The results concluded that among all the treatments tested Neem seed kernel extract has 53.0% suppression of radial growth of fungus which is followed by Neem oil with a mean suppression value of 43.8%.

Yasmin (2016) conducted an experiment to investigate the effectiveness of three botanical extracts and they reported that garlic, ginger and neem at different concentrations reduced the mycelial growth of *Bipolaris sorokiniana, Fusarium oxysporum* and *Sclerotium rolfsii* significantly ($p<0.01$).

2.7.3. Use of Black Cumin seed as bio-fungicide

Hossain *et al*. (1997) reported that *Nigella sativa* showed positive antifungal activity in reducing the pathogenicity of *Bipolaris sorokiniana* of wheat leaves.

Khatun (2018) surveyed on wheat blast and morphological characterization and *in-vitro* management of *Magnaporthe oryzae triticum* through botanicals. Result showed that *Nigella sativa* (Black cumin seeds) extracts @ (1:1 w/v) concentration found the one of the most effective botanicals to reduce mycelial growth of *M. oryzae triticum* under *in-vitro* condition.

Nazifa *et al.* (2021) reported that cumin seed 1:1 (w/v) inhibit 59.29% mycelial radial growth of *Magnaporthe oryzae oryza* at 7 DAI as compared to control. They also reported that among eight botanicals cumin seed 1:4 (w/v) inhibited 11.45% mycelial radial growth of *Magnaporthe oryzae oryza* at 14 DAI as compared to control.

Zohura *et al.* (2018) conducted research to evaluate the efficacy of twelve plant extracts and two fungicides against *Magnaporthe oryzae* Pathotype *triticum* (MoT) which is responsible for wheat blast disease. Research result concluded that black cumin seed extracts inhibit 90 mycelial growths at 10 days after inoculation.

2.7.4. Use of Garlic clove as bio-fungicide

Ahmed and Sultana (1984) observed that bulb extract of garlic was most effective against major seed borne pathogen of jute viz. *Macrophomina phaseolina*, *Botryodiplodia thebromae* and *Collectotrichum corchori*. They also reported that garlic clove extract successfully inhibited spore germination and mycelial growth of fungus.

Alice and Rao (1987) conducted an experiment where they evaluated 31 plant extracts *in vitro* against *Drechslera oryzae* in rice. They found that maximum inhibition of *D. oryzae* was obtained with *Mentha piperita* followed by *Piper nigrum* seed extract and *Allium sativum* extract.

Alice and Rao (1987) reported that garlic extracts have significant effect in controlling seed borne fungus *Drechslera oryzae* and increased the germination ability of the treated seeds.

Avasthi *et al.* (2010) tested eight commonly used spices *Syzygium aromaticum*, *Cinnamonum zeylanicum*, *Zingiber officinale*, *Murraya koenigii*, *Piper nigrum*, *Trachyspermum ammi*, *Allium sativum* and *Allium cepa* for *in vitro* antifungal activity on *Aspergillus niger*, a causative agent of different destructive disease. Out of eight plant materials used, five showed significant antifungal activity against the test pathogen by poisoned food technique. *Syzygium aromaticum* and *Allium sativum* showed 100% inhibition of mycelial growth at 20% concentration.

Bhagat *et al.* (2014) reported that seed treatment + foliar spray of freshly prepared garlic bulb extract has resulted into the reduction of *Alternaria* blight (35.6 %), white rust (50.4 %), powdery mildew (67.7 %) and *Sclerotinia* rot (80.3 %) in mustard with 27.3 % increase in yield over untreated control.

Das *et al.* (2018) studied *in vitro* efficacy of some antifungal plant extracts and reported that the aqueous extract of 15% concentration of *Allium sativum* reduced 100% mycelial growth of *Phomopsis vexans*.

Fiona *et al.* (2005) observed that allicin from garlic successfully inhibited the growth and infection of *Magnaporthe oryzae*.

Grozav and Foarce (2005) reported that garlic extract treatment of wheat seeds significantly reduced the incidence of seed-borne fungi, increased seed germination, the number of healthy seedlings and vigor index.

Hajano *et al.* (2012) conducted an in-vitro evaluation of fungicides, plant extracts and bio-control agents against rice blast pathogen *magnaporthe oryzae* couch. They reported that extracts of garlic (*Allium sativum* L.) at higher dose completely inhibited the mycelial growth of *magnaporthe oryzae*.

Hassan *et al.* (2005) reported that garlic extarcts completely controlled the intensity of *B. sorokiniana* and *Fusarium* spp. after the treatment of wheat seeds.

Islam (2018) reported that garlic bulb extract could be used as eco-friendly approach for controlling anthracnose disease of Aloevera (*Aloe vera* L.) caused by *Colletotrichum gloeosporioides*.

Jadeja (2003) reported that 10% extract of garlic clove inhibited the growth of *Phomopsis vexans* causing stem and branch blight in eggplant.

Lakshmonan et al. (1990) reported that garlic clove extract was most effective against *Corynespora cassiicola*. They also stated that garlic clove extracts inhibit mycelial growth and spore germination of *Corynespora cassiicola*.

Martínez (2012) reported that garlic (*Allium sativum* L.) contain volatile antimicrobial substances which inhibit *Aspergillus flavus*, *A. parasiticus*, *Candida albicans*, *Cryptococcus*, *Penicillium*, *Rhodotorula*, *Saccharomyces*, *Torulopsis* and *Trichosporon* species. The main compounds obtained from this extract is allicin (diallylthiosulfinate; thio-2-propene-1-sulfinic acid-S-allyl ester).

Miah *et al.* (1990) conducted an investigation to find out the efficacy of eight different plant extracts against seed borne fungi of rice. In this investigation they soaked rice seeds for eight hours with plant extracts. Results concluded that Extracts of *Allium sativum* effective against seed borne fungi.

Nashwa and Abo-Elyousr (2012) evaluated six plant extracts against the early blight disease of tomato plants under greenhouse and field conditions. They reported that during in vitro condition 5% concentration of *Allium sativum* caused 42.2% reduction of mycelial growth of *Alternaria solani* and in greenhouse 5% concentration of *Allium sativum* reduced 46.1% disease severity.

Parvin *et al.* (2016) conducted an investigation to determine the effect of botanicals on radial mycelial growth of *Sclerotium rolfsii in-vitro*. Experiment result concluded that garlic extracts showed profound and significant effect on reduction of radial mycelial growth of the fungus.

Perello *et al.* (2012) carried out *In vitro* studies to investigate the inhibitory effect of allicin in garlic juice on hyphal growth and spore germination of *Drechslera tritici-repetis*, *Bipolaris sorokiniana* and *Septoria tritici*. They reported that an amount of 53 µg per disc, allicin inhibited *B. sorokiniana* radial growth by 63% and growth of *D. triticirepentis* by 67%. They also reported that **a**llicin at 20 to 60 µg/ml caused a reduction of more than 50% in the germination of spores of *B. sorokiniana*, *S. tritici* and *D. tritici-repentis* and at 10 µg/ml, allicin caused morphological abnormalities in hyphae and conidia of *D. tritici-repentis* and *B. sorokiniana*.

Rahman (2007) conducted an experiment to find out the efficacy of seed treatment with plant extracts on leaf blight (*Bipolaris sorokiniana*) development and yield of wheat. In this experiment 33 plant species extract was evaluated and among them 13 species extract showed promising result against *Bipolaris sorokiniana*. Experiment result reported that second lowest severity of flag leaf and penultimate leaf blight in every stage observed when seeds were treated with garlic clove extract.

Slusarenko *et al.* (2008) completed a case study to increase awareness of the potential for developing plant protection strategies based on natural products. They reported that the volatile antimicrobial substance allicin (di-allyl-thio-sulphinate) is produced in garlic effectively controlled seed-borne *Alternaria spp*. in carrot, *Phytophthora* leaf blight of tomato and tuber blight of potato as well as *Magnaporthe* on rice and downy mildew of *Arabidopsis*.

Suharti *et al.* (2020) studied the effect of plant extracts and chemical fungicide on viability and percentage of seed-borne fungal infection on calliandra (*Calliandra callothyrsus*) seed. They reported that garlic extracts reduced 8.67% fungal infections.

Yeni (2011) evaluated antifungal effects of extracts of *Allium sativum* and *Nicotiana tobacum* on rot causing organisms on yam: *Aspergillus niger*, *Fusarium oxysporum*, *Rhizopus stolonifer*, *Botryodiplodia theobromae*, *Aspergillus flavus* and *Fusarium solani*. Result concluded that 76.66% inhibition of *Botryodiplodia theobromae* was obtained using 80% aqueous extract of *Allium sativum*, 6.66% inhibition of *Botryodiplodia theobromae* was obtained using 30% ethanol on *Allium sativum*.

Zohura *et al.* (2018) conducted a research 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. They reported that the highest percentage of mycelial inhibition (93.75%) was recorded in case of four plant extracts namely Tulsi, Mehendi, Datura and Garlic. They also reported that *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 during *in vitro* test.

CHAPTER III

MATERIALS AND METHODS

This section includes the detail of the materials that have been used to conduct the experiment and the procedures that have been followed to carry out the experiment concerned.

3.1. Laboratory experiment

3.1.1. Experimental site

Laboratory investigations were conducted in the Advanced Nematology Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207, Bangladesh.

3.1.2. Experimental period

The experiment was carried out during the period from August to October 2020.

3.1.3. Test materials and sample collection

Infected seed was collected from blast infected wheat growing region, Kushtia, Bangladesh. Three varieties *viz*. Prodip, BARI Gom-26 and BARI Gom-28.

3.1.4. Sterilization of materials and lab equipment

Glass wares *viz.* petri dishes; conical flask, glass tube, glass rod etc. were sterilized in hot air oven at 165°C for 3 hours. PDA media and distilled water were sterilized in an autoclave following the method (Hazra, 1988) at 121°C under 15 pound per square inch (psi) for 20 minutes. 0.1 % sodium hypochlorite (NaOCl), 70% ethanol were used for surface sterilization of collected wheat seed. Hexisol (alcohol-based sanitizer) was used for hand sanitization. Inoculation chamber was sterilized and prepared by spraying 70% ethanol in the inner surface of the Laminar Air Flow (LAF) cabinet. Rectified spirit was used in lamp for burning other equipment like inoculation-needle tip, forceps. During culture transfer, they were sterilized in flame to avoid contamination. All the materials and equipment except pathogen culture were further sterilized under UV light inside the Laminar Air Flow (LAF) cabinet.

3.1.5. Preparation of culture media

The standard Potato Dextrose Agar (PDA) media (200g of peeled potatoes, 20 g of dextrose, and 20 g of agar and 1000 ml of distilled water) and Oat Meal Agar (OMA) media were used in *in vitro* experiment. Cleaned and peeled potato tubers were sliced into pieces. Then the pieces were boiled in distilled water to collect the extract by sieving with a fine piece of cloth. Dextrose and agar were dissolved in the potato extract and the volume was made up to 1000 ml by adding distilled water. After preparation, the media was poured into 500 ml Erlenmeyer flasks, plugged with cotton and wrapped with aluminum foil. The flasks containing media were sterilized in the autoclave at 121°C under 15 pound per square inch (psi) for 20 minutes. The media were acidified with 30 drops of lactic acid per 250 ml medium to inhibit the growth of bacteria. 20 ml of medium was poured into each petri dish (9 cm diameter) inside Laminar Air Flow (LAF) with proper cautions and then allowed to solidify.

In Oat Meal Agar (Oat flakes 30g, Agar-agar 20 g, Distilled water (to make up) 1000.00 ml). First oat flakes were boiled with 500 ml of distilled water for 30 min and filtered through muslin cloth. Agar was melted in 500 ml water separately. Both the solutions were mixed thoroughly and sterilized as described previously.

3.1.6. Seed health study, determination of seed germination and incidence of seed borne fungi in the collected seed samples

Diseased seeds of wheat cultivars infected with pathogen were surface sterilized with 1% sodium hypochlorite for 1 minute followed by 3 times washes with sterile distilled water. Then the infected seeds were placed in Petri dishes lined with moist filter papers and it was incubated at 26+1^{o}C for 24 hours to encourage sporulation. After incubation, these infected seeds were examined under stereo-dissecting microscope. Abundant sporulation was observed from in and around the lesions with grey, dense and bushy appearance. Data on seed germination and incidence of fungi on seeds were recorded at 10 days of incubation.

3.1.7. Isolation and identification of causal organism of blast disease of wheat

The water agar (Agar 20 g with 1000 ml distilled water), and potato dextrose agar media (PDA) were used for the isolation of blast pathogen. A sterile moistened needle was used to pick out single conidia by the needle across the sporulating lesion. The conidia were placed on water agar. After 12 hours, mycelium was visible in petri dish and it then hyphal tip was placed in potato dextrose agar media plates containing Streptomycin (40 mg L^{-1}) and pure culture of *Magnoporthe oryzae triticum* was prepared by incubating there in 26+1°C. The marginal mycelial growth that developed subsequently was picked-up aseptically for sub-culturing until pure culture of *Magnoporthe oryzae triticum* was obtained. The pure culture was maintained by sub culturing at an interval every 15 days and preserved at low temperature (4°C) in refrigerator.

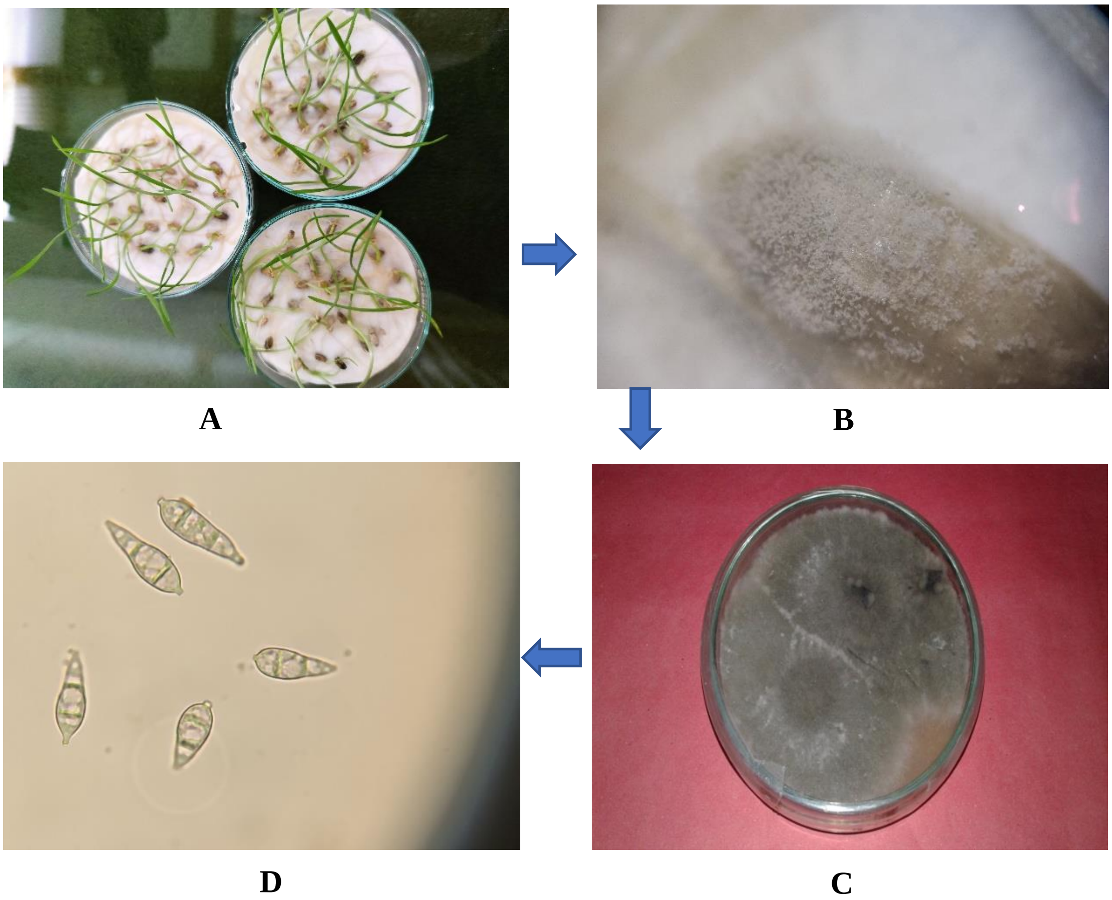


Figure 1. Flow chart of isolation, identification and culture of *Magnaporthe oryzae triticum* on Oat Meal Agar media. A. Incubation of infected seed on moist chamber; B. Habit characteristics of *Magnaporthe oryzae triticum* on seed observed under steriomicroscope (x40); C. Pure culture of MoT on OMA and D. Conidia of MoT under compound microscope (x60)

For sporulation, oat meal agar (40g of oats, 5g of sucrose, 20 g of agar and 1000ml of distilled water) plates were used. After placing the block of mycelium of *Magnoporthe. oryzae triticum*, plates were incubated at 26+1°C for about 10 to 15 days with alternate 12 hours darkness and 12 hours light for sporulation. After conidia production in OMA (Oat Meal Agar) plates, the conidia of *Magnoporthe oryzae tricicum* were checked under compound microscope. Identification of the pathogen was carried out according to the cultural and morphological characteristics as described by (Agrawal *et al.*, 1989 and Mew *et al.*, 2002).

3.1.8. Pathogenicity tests for *M. oryzae triticum* isolates

The pathogenicity test of the isolates was done for further confirmation of pathogenic isolates of wheat blast pathogen. The pathogenicity of *M. oryzae triticum* isolates was confirmed by Koch's postulates using the method of Chevalier *et al.,* 1991. The pot was filled using previously sterilized field soil. Disinfected viable seeds of BARI Gom-24 (Pradip) susceptible to MoT isolates were sown in pots with 10 seeds per pot. The plants were inoculated after germination, at the age of 3-4 leaves and the seedlings in each pot was sprayed with 40–50 ml of spore suspension adjusted to 105 spores ml^{-1} with the help of hemocytometer. Atomizer sprayer was rinsed with 95% Ethanol and then washed with sterile distilled water and used for spraying (Hans *et al.,* 2003). The conidial suspensions were sprayed on to the wheat seedlings until runoff while water was used for spraying the control treatment. Inoculated pots were covered with moistered polythene bags and incubated at $26+^{0}C$ for 7 days. Periodical observations were made for the development of symptoms on the leaves starting 7 days after inoculation. Experiments were done with three replications. The fungus was re-isolated from the artificially inoculated wheat seedlings leaves showing typical blast symptom.

3.1.9. Maintenance of isolates *Magnaporthe oryzae triticum*

The fungus was sub-cultured on OMA media and kept at $26\pm1^{O}C$ for 15 days. Subsequent, subculturing of isolates was done at an interval of 30 days. Such isolates were stored in a refrigerator at -20^{0} C and revived monthly.

3.2. Field Experiment: Development of eco- friendly management for blast disease of wheat

3.2.1. Experimental site

The experiment was conducted in the field laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207 (**Appendix I).**

3.2.2. Climatic condition

The experimental site has a subtropical climate. It is mostly rained on by the South West monsoon (May-October) and the winter season (November-February). The meteorological data for the trial period was obtained from the Sher-e Bangla Nagar Meteorological Station in Dhaka-1207. During the crop growing period in 2020, the maximum temperature varied from 15°C to 35°C, with an average of 28.50°C, while the lowest temperature ranged from 10°C to 24°C, with an average of 17.30°C. The average relative humidity was between 57 and 74 percent. During the crop growing season, the total rainfall received was 302 mm over 27 wet days.

3.2.3. Soil

The research field's soil comes from "The Modhupur Tract," AEZ – 28, and is mildly acidic due to low organic matter concentration. During the experimental time, the experimental area was above flood level and had enough sunlight, as well as an irrigation and drainage system. Soil samples were taken from the experimental field at a depth of 0-15 cm, and soil and plant analysis were performed at the Soil Resources Development Institute (SRDI) in Dhaka. The experimental plot was made out of high terrain with a pH of 5.6. **Appendix II** lists the physical parameters and nutritional condition of the soil and plants in the experimental plot.

3.2.4. Experimental period

The experiment was conducted during November 2020 to April 2021.

3.2.5. Test materials used

"Prodip" wheat variety was used for field experiment because among three test varieties "Prodip" showed maximum % seed borne infection of MoT.

3.2.6. Land Preparation

A piece of medium high land having well drainage system was selected. The soil texture of the experimental site was loam to clay loam belonging to AEZ-28: Madhupur Tract . Weeds, small bricks, stone, trashes were removed from the field. Two or three ploughing followed by cross ploughing and laddering was performed to make to soil loose and friable. Sevin 85 SP was used to remove ants and termite from soil. The land was levelled properly. Plots for seed showing were made.

3.2.7. Layout

The field was divided into three blocks each of which representing a replication. The unit plot size was 2.50 m × 2 m and plot to plot distance was 1 m and block to block distance was 1 meter (**Appendix III**).

3.2.8. Treatments

Eco-friendly management components along with control were selected as treatment. Those are given bellow:

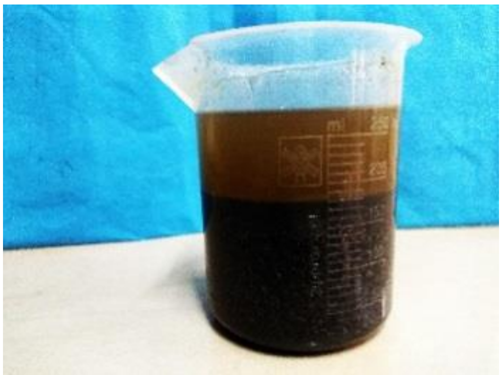
T_1 = Control
T_2 = *Allium sativum* (Garlic clove extracts, 1:5 w/v)
T_3 = *Aloe vera* (Allovera leaf extracts,1:5 w/v)
T_4 = *Nigella sativa* (Black cumin seed extracts, 1:5 w/v)
T_5 = *Azadirachta indica* (Neem leaf extracts, 1:5 w/v)
T_6 = Nativo 75 WG (0.1%)
T_7 = Provax 200 WP (0.3%)

3.2.9. Preparation of Eco-friendly fungicide (Botanicals)

The extracts were prepared by using the method of Ashrafuzzaman and Hossain (1992). For preparation of extracts, collected leaves and bulbs were weighted in an electric balance and then washed in the water. After washing the big leaves were cut into small pieces. For getting extract, weighted plant parts were blended in an electric blender and then distilled water was added into the jug of the blender. The pulverized mass was squeezed through 3 folds of fine cotton cloth. For getting 1:5

(w/v) ratio 500 ml of distilled water was added with 100 g plant parts. Botanical solutions were also prepared in the day of spraying.

A

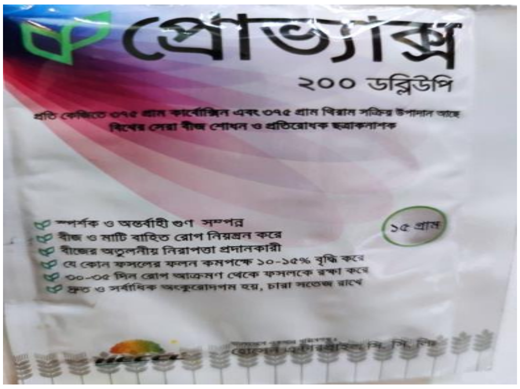


B

C

D

E



F

Figure 2. Treatments used in controlling wheat blast disease caused by *Magnaporthe oryzae triticum* A. Extracts of *Allium sativum* B. Extracts of *Nigella sativa* C. Extracts of *Aloe vera* D. Extracts of *Azadirachta indica* E. Nativo 75 WG and F. Provax 200 WP

3.2.10. Preparation of fungicides

Fungicides prepared according to the user guide provided with fungicide.

3.2.11. Application of fertilizers and manures

All the fertilizers were applied at the rate of BARI recommended dose as 150kg ha TSP, 50 kg ha MOP, 120 kg ha Gypsum. Nitrogen fertilizers were applied as per treatments in each plot. Fertilizers other than nitrogen were given during land preparation. The whole amount of all the fertilizers except urea were applied at the time of land preparation and thoroughly incorporated with soil with the help of a spade.

3.2.12. Seed sowing

Seeds were sown on November 26, 2020, in 20 cm apart rows maimtained by specially made iron hand tine. The seed rate was 120 kg ha^{-1}. After sowing, the seeds were covered with soil and slightly pressed by hands.

3.2.13. Application of fungicides

At recommended doses, the solution of fungicides was prepared by mixing thoroughly with requisite quantity of normal plain water. Solution of Provax 200 WP was used for seed treatment. Nativo 75 WG solution was sprayed on 28 January, 2021. To avoid the drifting of the fungicide during application, spraying was done very carefully, specially observing air motion. A control treatment was maintained in each block where spraying was done with plain water only.

3.2.14. Application of botanical extracts

Botanicals were sprayed in research plots on 28 January, 2021. To avoid the drifting of the botanicals during application, spraying was done very carefully, specially observing air motion. A control treatment was maintained in each block where spraying was done with plain water only.

3.2.15. Intercultural Operation

Different intercultural operations were done as follows.

3.2.15.1. Thinning

Emergence of seedling was completed within 10 days after sowing. Overcrowded seedlings were thinned out for two times. First thinning was done after 15 days of sowing which is done to remove unhealthy and lineless seedlings. The second thinning was done 15 days after first thinning keeping healthy seedlings.

3.2.15.2. Irrigation

The experimental plots required three irrigations during the crop growth season and sometimes drainages were done at the time of heavy irrigation. The first irrigation was done at 20 DAS, crown root initiation stage. Second irrigation was provided at 50 DAS which is the maximum tillering stage of wheat and the last irrigation was done a 72 DAS, grain filling stage. Proper drainage system was also made for draining out excess water.

3.2.15.3. Weeding

During plant growth period two hand weeding were done. First weeding was done at 30 days after seed sowing followed by second weeding at 20 days after first weeding.

3.2.15.4. Netting

Netting was done to protect the seeds from birds.

3.2.15.5. Harvest and post-harvest operation

The maturity of crop was determined when 85% to 90% of the grains become golden yellow in color. Harvesting was done at March, 2021. From the center of each plot 1 m^2 was harvested to assess yield of individual treatment and converted into ton ha^{-1}. The harvested crop of each plot was bundled separately, tagged properly and brought to threshing floor. The bundles were dried in open sunshine, threshed and then grains were cleaned properly. The grain and straw weights for each experimental plot were recorded after proper drying in sun.

3.2.16. Data collection

Data on the following parameter was recorded:

1. Panicle blast incidence (%) at milking stage, soft dough stage and hard dough stage
2. Panicle blast severity at milking stage, soft dough stage and hard dough stage
3. Weight of seeds per plot (g)
4. 1000 seeds weight (g)

5. Spike length (cm)
6. Spikelets spike^{-1}
7. Yield (t ha^{-1})

3.2.17. Determination of panicle blast incidence (%) and severity (%)

Incidence of panicle blast was calculated in the number of proportions of the plant units diseased in relation to the total number of units examined. Plant units mean the leaves, stems, panicles, etc. that show any symptoms. Disease incidence was calculated by following formula:

$$\text{Disease incidence (\%)} = \frac{\text{Numbers of infected plant} \times 100}{\text{Numbers of inspected plant}}$$

Panicle blast severity was determined using the scale proposed by Trindade *et al*., (2006). It was based according to the point at which the pathogen had penetrated the rachis and affected the length of the spike. The score 0 referred to no visual symptoms, 1 for 25 % of the spike showing symptoms; 2 for 50%, 3 for 75% and 4 for 100% length of spike affected.

3.2.18. Spike length (cm)

Spike length were counted from five plants from basal node of the rachis to apex of each spike and then averaged

3.2.19. Number of spikelets spike^{-1}

Number of spikelets were counted from 5 spikes and averaged to determine the number spikelets $^{-1}$.

3.2.20. Weight of 1000 grains

One thousand cleaned dried grains were counted randomly from each plot and weighed by using a digital electric balance when the grains retained 12% moisture and the mean weight was expressed in gram.

3.2.21. Grain yield (t ha^{-1})

Grain yield was determined from the central 1 m^2 area of each plot and expressed as t ha^{-1} on 12% moisture basis. Grain moisture content was measured by using a digital moisture tester.

3.2.22. Seed health study of harvested seeds

Seed health study of harvested seeds was done following the methods as described previously in 3.1.6.

3.2.23. Design of the experiment

Field experiment was conducted following by Randomized Complete Block Design (RCBD) with three replications.

3.2.24. Statistical analysis of data

The relevant data were statistically analyzed using analysis of variance to find out the variation of results from experimental treatments by Statistics 10 software. Treaments means were compared by DMRT.

CHAPTER IV

RESULTS AND DISCUSSION

The findings of environmentally friendly wheat foliar disease treatment were given and documented in this chapter. The analysis of variance of data on disease incidence (%) at milking stage, soft dough stage, hard dough stage; disease severity at milking stage, soft dough stage, hard dough stage; grain weight per plot (g); weight of thousands grain (g); Panicle length (cm), spikelets spike^{-1}; yield (t ha^{-1}) obtained from the current experiment were presented and discussed in this chapter. The results and possible interpretations have been organized under the heading below for ease of debate, comprehension and understanding.

4.1. Germination of wheat cultivars and disease Incidence of wheat blast across wheat cultivars

Depending on the cultivar, the seed germination and the incidence of blast disease differed. Farmers' favorite cultivar is BARI Gom-24 (Pradip), however other cultivars such as BARI Gom-26, BARI Gom-28, and others were recently released and planted in a few farmer fields. In this experiment, cultivars like BARI Gom-24 (Pradip) had the maximum seed germination and % MoT incidence, whereas cultivars like BARI Gom-26 and BARI Gom-28 had the lowest %MoT incidence (Table 2).

Table 1. Germination of wheat cultivars and %incidence of seed borne fungi on wheat seeds

Variety	Germination percentage (%)	Incidence of *Magnaporthe oryzae* (%)	Incidence of *Bipolaris sorokiniana* (%)	Incidence of *Alternaria triticina* (%)	Incidence of *Aspergillus niger* (%)
Prodip	93	30	12	15	14
BARI Gom-26	90	0.1	13	11	12
BARI Gom-28	92	0.8	13	10	11

4.2. Effects of different control measures on incidence (%) and severity of panicle blast of wheat at different grown stages

4.2.1. Effects of different control measures on blast disease incidence (%) of wheat at milking stage

Disease incidence (%) indicted the presence of disease in investigated area. Different treatments showed statistically significant variation on blast disease incidence of wheat at milking stage (Figure 3). Among different treatments highest blast disease incidence (13.13 %) was observed with T_1 (control) treatment which were statistically similar with T_2, T_3, T_4 treatments. Lowest disease incidence (7.86 %) was observed with T_7 (Provax 200 WP) treatment which showed statistically identical result with T_5 (Neem leaf extracts) treatment. Results concluded that chemical fungicide showed statistically similar results with eco-friendly botanicals. Martinez (2012) and Yoon *et al.* (2013) reported that natural products which are safe for the environment and have low toxicity to living organisms are gaining interest as important sources for the development of fungicides, and these may serve as effective substitutes for synthetic fungicides. Agbowuro *et al.* (2020) and Rijal and Devkota (2020) supported the findings of this experiment and stated that neem extract 4ml/15ml best for reduction of blast incidence and mycelial growth of *Magnaporthe oryzae triticum*.

4.2.2. Effects of different control measures on blast disease severity of wheat at milking stage

Different treatments showed statistically significant variation on panicle blast severity of wheat at milking stage (Figure 3). Among different treatments highest blast disease severity score (1.97) was observed with T_1 (control) treatment followed by T_4 treatment which showed 1.60 disease severity at milking stage. Lowest disease severity 1.03 was observed with T_7 (Provax 200 WP) treatment which showed statistically identical result with T_5 (Neem leaf extracts) treatment. T_5 treatment showed 1.13 blast disease severity at milking stage. It has been observed that chemical fungicide showed statistically similar results with ecofriendly botanical treatment. The severity of the disease during the milking stage resulted in poor wheat grain development. Natural compounds that are safe for the environment and have low toxicity to living animals are gaining interest as important sources for the manufacture of fungicides, according to Yoon *et al.* (2013), and they may serve as viable alternatives to synthetic fungicides. Siresha and Venkateswarlu (2013) backed up the findings of this study, claiming that ecofriendly botanicals contain active therapeutic characteristics that can aid to lower the severity of wheat blast disease.

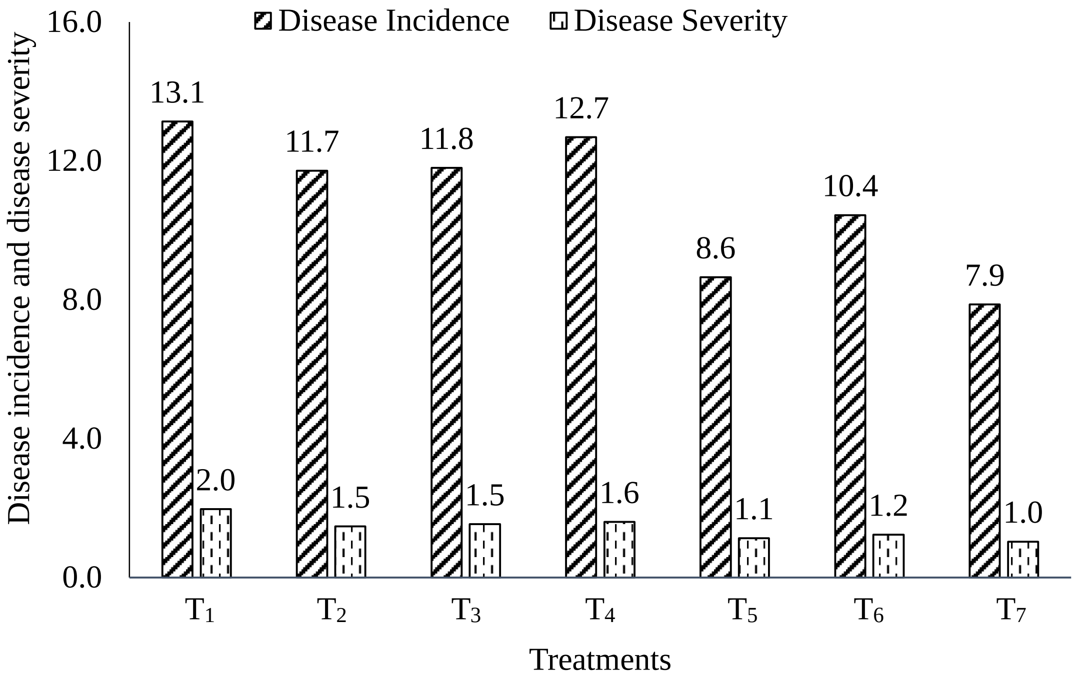


Figure 3. Effects of different control measures on disease incidence (%) and disease severity of wheat blast at milking stage. Where T_1=Control, T_2=Garlic clove extract, T_3=Aloe vera leaf extract, T_4=Black cumin seed extract, T_5=Neem leaf extract, T_6=Nativo 75 WG and T_7=Provax 200 WP

4.2.3. Effects of different control measures on blast disease incidence (%) of wheat at soft dough stage

At the soft dough stage, several treatments exhibited statistically significant differences in blast disease incidence of wheat (Figure 4). Among different treatments highest blast disease incidence (19.73 %) was observed with T_1 (control) treatment followed by T_4 treatment. Lowest disease incidence 9.86 % was observed with T_7 (Provax 20WP) treatment which showed statistically identical results with T_5 (Neem leaf extracts) treatment (10.10%). It has been observed that chemical fungicide showed statistically similar results with ecofriendly botanicals used for management of blast disease of wheat. According to Hajano *et al.* (2012), the widespread use of fungicides on crops can harm humans, plants, and beneficial microorganisms, as well as promote fungicide resistance in pathogens and persistent toxicity in plant components. Some botanical pesticides and bio-control agents, on the other hand, have shown to be extremely safe and have no negative effects on the environment. The findings of this experiment were corroborated by Agbowuro *et al.* (2020) and Rijal and Devkota (2020), who indicated that neem extract 4ml/15ml was the best for reducing blast incidence and mycelial development of *Magnaporthe oryzae triticum*.

4.2.4. Effects of different control measures on blast disease severity of wheat at soft dough stage

Several treatments had statistically significant effect on panicle blast severity of wheat during the soft dough stage (Figure 4). T_1 (control) treatment had the highest blast disease severity of 2.33, followed by T_4 treatment. T_7 (Provax 200 WP) treatment had the lowest disease severity of 1.23, which was statistically equal to T_5 (Neem leaf extracts) treatment. T_5 treatment reduced blast disease severity at 1.27. It has been observed that chemical fungicides had statistically equivalent outcomes to ecofriendly botanicals for the treatment of wheat blast disease.

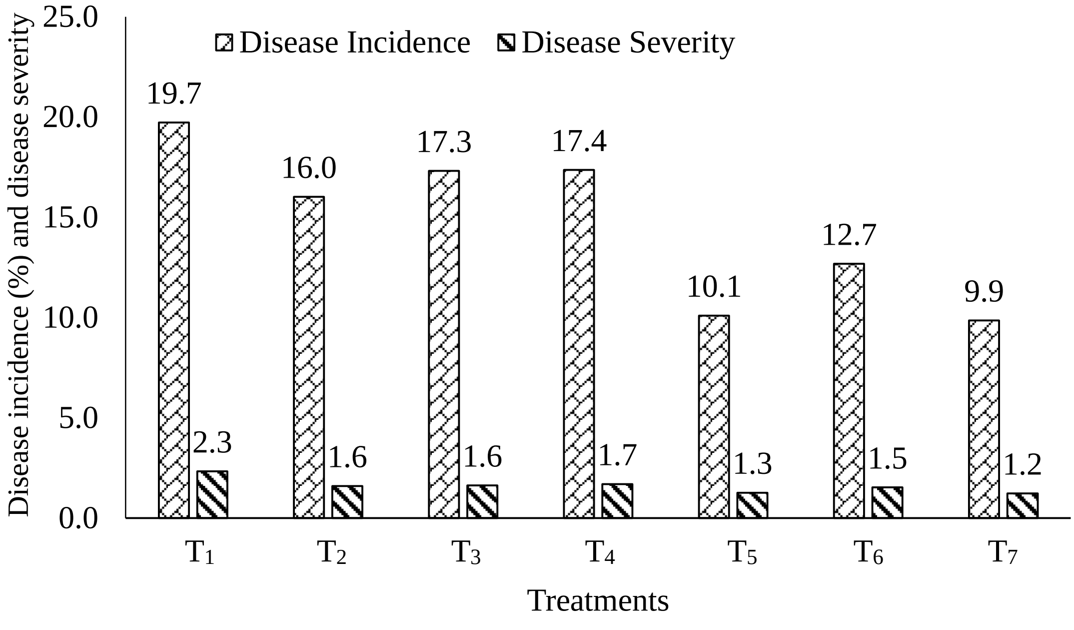


Figure 4. Effects of different control measures on disease incidence (%) and disease severity of wheat blast at soft dough stage. Where T_1=Control, T_2=Garlic clove extract, T_3=Aloe vera leaf extract, T_4=Black cumin seed extract, T_5=Neem leaf extract, T_6=Nativo 75 WG and T_7=Provax 200 WP

The extensive use of fungicides on crops, according to Hajano *et al.* (2012), can damage humans, plants, and beneficial microbes, as well as induce fungicide resistance in pathogens and permanent toxicity in plant components. On the other hand, several botanical pesticides and bio-control agents have been found to be exceedingly harmless and have no detrimental environmental consequences. Agbowuro *et al.* (2020) and Rijal and Devkota (2020) confirmed the outcomes of this study, stating that neem extract 4ml/15ml was the best for lowering blast incidence and severity of *Magnaporthe oryzae triticum*

4.2.5. Effects of different control measures on blast disease incidence (%) of wheat at hard dough stage

On blast disease incidence (%) of wheat at the hard dough stage, there was statistically significant variance among different ecofriendly management (Figure 5). At hard dough stage T_1 (control) treatment had the greatest blast disease incidence (21.12 %), followed by T_4 treatment. T_7 (Provax 200 WP) treatment had the lowest disease incidence (10.19 %), which was statistically equal to T_5 (Neem leaf extracts) treatment (10.44%). It has been observed that chemical fungicides had statistically equivalent outcomes to ecofriendly botanicals for the treatment of wheat blast disease. Yoon *et al.* (2013) highlighted that natural compound which are safe for the environment and have minimal toxicity to living creatures are gaining interest as major sources for the creation of fungicides, and they may serve as viable alternatives for synthetic fungicides. Shabana *et al.* (2016), Siresha and Venkateswarlu (2013) supported the results of this experiment and reported that ecofriendly botanicals have active medicinal properties which can help to reduce the blast disease incidence (%) of wheat.

4.2.6. Effects of different control measures on blast disease severity of wheat at hard dough stage

During the hard dough stage of wheat some treatments exhibited statistically significant differences on panicle blast severity of wheat (Figure 5). Among different treatments highest disease severity 2.63 observed with T_1 treatment (Control) at hard dough stage of wheat. Lowest disease severity (1.63) was observed with T_7 treatment (Provax 200 WP) which was statistically identical with disease severity (1.73) showed by T_5 treatment at hard dough stage of wheat. It has been observed that ecofriendly management can provide similar results for controlling wheat blast disease like chemical fungicides which are harmful for environment. Yoon *et. al.* (2013) reported that terpenes, phenols, alcohols, alkaloids, tannins and other secondary metabolites found in botanicals induce toxicity to fungal cell walls, cell membranes and cell organelles. These metabolites also inhibit spore germination, mycelial development, germ tube elongation, delayed sporulation and also inhibit production of important enzymes, DNA and protein synthesis. The results of this investigation were corroborated by Shabana *et al.* (2016), Siresha and Venkateswarlu (2013), Hajano *et al.* (2012) who claimed that ecofriendly botanicals contain active therapeutic qualities that can aid to lower the blast disease severity of wheat.

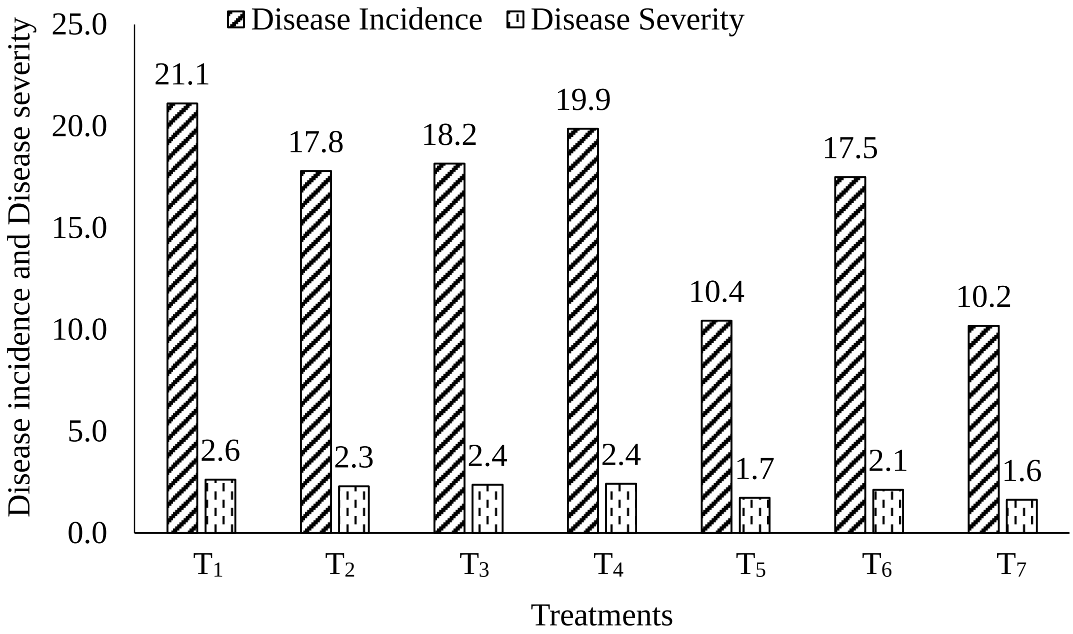


Figure 5. Effects of different control measures on disease incidence (%) and disease severity of wheat blast at hard dough stage. Where T_1=Control, T_2=Garlic clove extract, T_3=Aloe vera leaf extract, T_4=Black cumin seed extract, T_5=Neem leaf extract, T_6=Nativo 75 WG and T_7=Provax 200 WP

4.3. Effects of different control measures on growth and yield of wheat

4.3.1. Effects of different control measures on spike length (cm) of wheat

Different treatments showed statistically significant difference on spike length (cm) of wheat (Table. 4). Highest spike length (17.9 cm) of wheat obtained with T_7 (Provax 200WP) treatment. Among different treatments T_2, T_3, T_4, T_5, T_6 showed statistically similar results. Lowest spike length (16.2 cm) of wheat obtained with T_1 (control) treatment. Yoon *et al.* (2013) toxicity of fungal cell walls, cell membranes, and cell organelles is caused by terpenes, phenols, alcohols, alkaloids, tannins, and other secondary metabolites found in botanicals. These metabolites also prevent spore germination, mycelial growth, germ tube elongation, delayed sporulation, and the creation of essential enzymes, DNA, and proteins. Ecofriendly botanicals reduced disease infestation in crop field which helps increase crop plant growth. Agbowuro *et al.* (2020) and Rijal and Devkota (2020) supported the findings of this experiment.

4.3.2. Effects of different control measures on spikelets spike $^{-1}$ of wheat

Wheat spikelets spike^{-1} differed statistically significantly across treatments (Table. 4). Highest spikelets spike^{-1} (21.1) was obtained when T_7 treatment used in field for controlling blast disease of wheat which showed statistically similar result when T_5 (Neem leaf extracts) used for controlling for blast disease of wheat. Lowest spikelets spike $^{-1}$ (17.1) was obtained from T_1 (control) treatment. Natural materials that are safe for the environment and have minimal toxicity to living creatures are gaining interest as major sources for the creation of fungicides, according to Martinez (2012) and Yoon *et al.* (2013), and they may serve as viable alternatives for synthetic fungicides. Ecofriendly botanicals decreased disease infestation in farming fields, allowing for greater crop plant growth and productivity. Rijal and Devkota (2020); Siresha and Venkateswarlu (2013); Ahmed *et al.* (2019); Keta *et al.* (2019); Hossain *et al*. (2005); Agarry *et al*. (2005); Nazifa *et al.* (2021) and Pandey (2015) supported the findings of this experiment and stated that botanicals help reduce disease severity in wheat and increase wheat growth.

4.3.3. Effects of different control measures on Grain weight per plot (g) of wheat

Grain weight per plot (gm) of wheat showed statistically significant variation with different botanical application for management of wheat blast disease (Table. 4). Highest grain weight per plot 1379.3 g followed by 1352 g obtained by application of T_7 (Provax 200WP) and T_5 (Neem leaf extract) treatment, respectively. There was no statistically significant difference among findings of T_7 and T_5 treatment. Lowest grain weight per plot 1056.0 g obtained with T_1 (control) treatment. According to Martinez (2012) and Yoon *et al.* (2013), organic resources that are safe for the environment and have low toxicity to living animals are gaining interest as key sources for the manufacture of fungicides, and they may serve as viable alternatives to synthetic fungicides. Experiment results also concluded that the use of environmentally friendly botanicals in farming areas reduced disease infestation, allowing for increased crop plant growth and output. Rijal and Devkota (2020); Siresha and Venkateswarlu (2013); Ahmed *et al.* (2019); Keta *et al.* (2019); Hossain *et al*. (2005); Agarry *et al*. (2005); Nazifa *et al.* (2021) and Pandey (2015) supported the investigation result and stated that botanicals help reduce disease severity in wheat and increase wheat production.

4.3.4. Effects of different control measures on weight of thousands grain (g) of wheat

Different control measures showed a positive effect on the weight of thousands grain (g) of wheat. There was a statistically significant relation among different treatments (Table. 4). The Highest weight of thousands grain 53.4 g of wheat obtained with T_7 (Provax 200WP) which was statistically identical with results of $T_{2,}$ $T_{3,}$ $T_{4,}$ T_5 and T_6 treatment. T_1 showed the lowest weight of thousands grain 48.2 gm. Weight of thousand seed is a genetical character which less impacted by the control measures. This investigation results concluded that all eco-friendly botanicals help to increase wheat yield which was statistically similar to yield obtained by using chemical fungicide for blast management. According to Martinez (2012) and Yoon *et al.* (2013), organic resources that are safe for the environment and have low toxicity to living animals are gaining interest as key sources for the manufacture of fungicides, and they may serve as viable alternatives to synthetic fungicides. Rijal and Devkota (2020); Siresha and Venkateswarlu (2013); Ahmed *et al.* (2019); Keta *et al.* (2019);

Hossain *et al*. (2005); Agarry *et al*. (2005); Nazifa *et al.* (2021) and Pandey (2015) supported the results of this experiment.

4.3.5. Effects of different control measures on Yield (t ha^{-1}) of wheat

Different control approaches have a favorable impact on wheat yield (t ha^{-1}). Wheat yield (t ha^{-1}) varied statistically significantly with different botanical treatments for wheat blast disease control (Table. 4). Highest yield 2.64 t ha^{-1} was obtained with Provax 200 WP treatment which was 28.9 % higher than that of control and was statistically similar with yield obtained with Neem leaf extract treatment (2.63 t ha^{-1}) which was 28.4 % higher than that of control. Lowest yield 2.05 t ha^{-1} when filed was maintained without any chemical or botanicals (T_1) for blast disease of wheat. Yoon *et al.* (2013) highlighted that natural compound which are safe for the environment and have minimal toxicity to living creatures are gaining interest as major sources for the creation of fungicides, and they may serve as viable alternatives for synthetic fungicides. The extensive use of fungicides on crops, according to Hajano *et al.* (2012), can damage humans, plants, and beneficial microbes, as well as induce fungicide resistance in pathogens and permanent toxicity in plant components. Rijal and Devkota (2020); Siresha and Venkateswarlu (2013); Ahmed *et al.* (2019); Keta *et al.* (2019); Hossain *et al*. (2005); Agarry *et al*. (2005); Nazifa *et al.* (2021) and Pandey (2015) supported the results of this experiment and stated that botanicals are same effective as chemical fungicide for controlling blast disease of wheat and help to maintain wheat production in blast infested area.

4.3.6. Effect of different control measures on association of MoT isolates on panicle and harvested seeds

After harvesting of crops, infected glume and seeds were separately incubated on moist blotter paper and observed under steriomicroscope. Habit characters of MoT isolate (conidia) was observed on glume but conidia was not seen on harvested seeds after incubation. The reason behind this is probably the environmental factors that not favoured during seed infection. Seed transmisssion nature of MoT isolate need further evaluation in controlled condition.

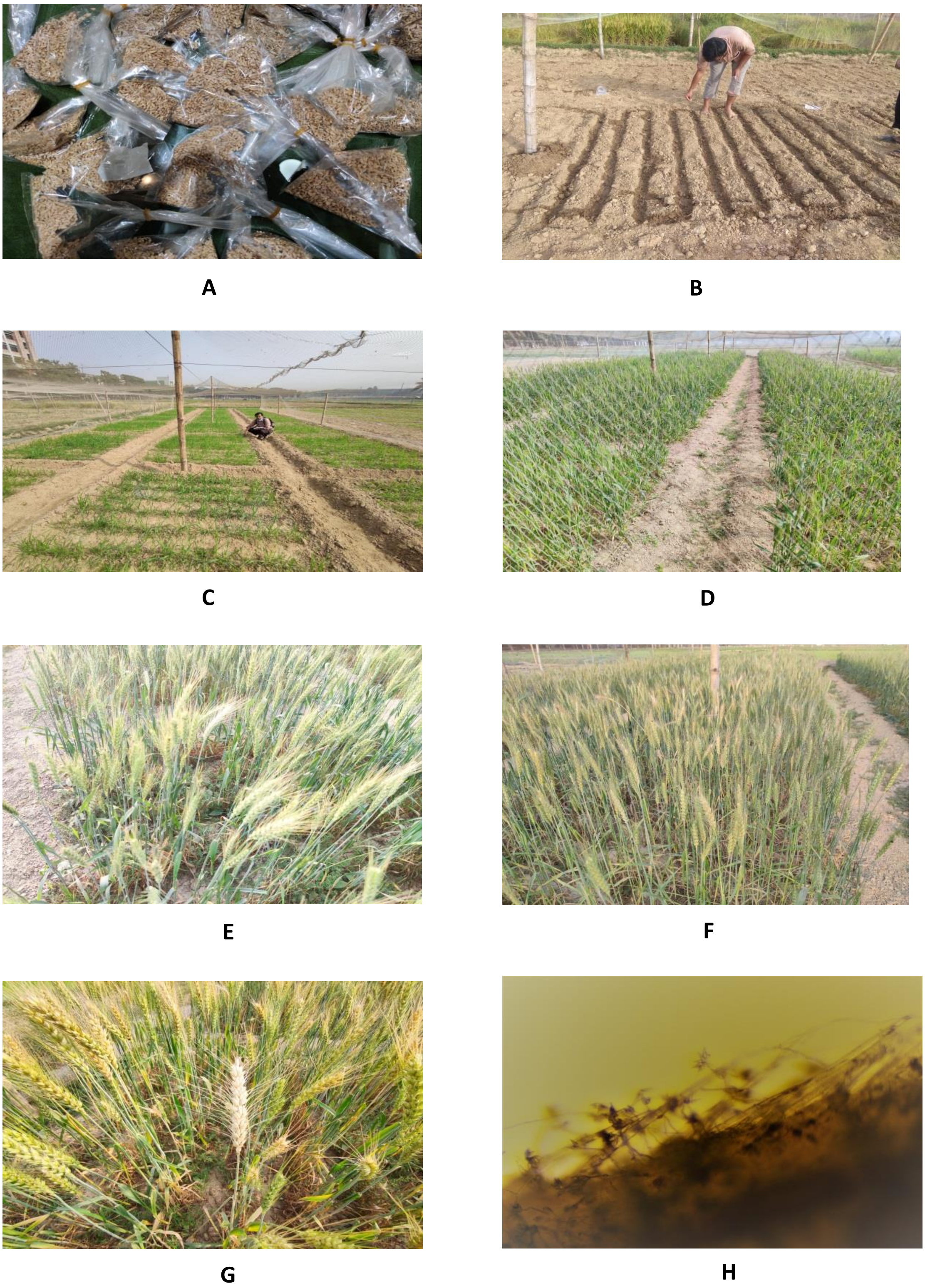


Figure 6. Experimental activities and field view. A. Collected seed sample; B. Seed sowing in the field; C. Field visit in seedling stage; D. Experimental wheat field during panicle initiation stage; E and F. Infected field; G. Bleached spike and H. MoT sporulation on nfected glume under stereomicroscope after harvest (x40)

Table 2. Effects of different control measures on growth and yield of wheat

Treatments	Panicle length (cm)	Spikelets per panicle	Grain weight per plot (g)	Weight of thousands grain (g)	Yield (tha^{-1})	Yield increase over control (%)
T_1	**16.2 c**	**17.1 d**	**1056.0 d**	**48.2 b**	**2.05 c**	--
T_2	16.9 bc	18.3 bc	1182.7 cd	**50.6 ab**	2.36 b	15.5
T_3	16.9 bc	18.2 bc	1182.0 cd	**50.1 ab**	2.31 b	13.0
T_4	16.5 bc	18.0 c	1106.0 cd	**50.1 ab**	2.25 bc	9.8
T_5	17.1 b	**20.9 a**	**1352.0 ab**	**52.9 ab**	**2.63 a**	28.4
T_6	17.0 bc	19.1 b	1222.3 bc	**51.0 ab**	2.37 b	15.6
T_7	**17.9 a**	**21.1 a**	**1379.3 a**	**53.4 a**	**2.64 a**	28.9
LSD $_{(0.05)}$	**0.83**	**1.02**	**152.15**	**4.71**	**0.22**	**--**
C.V. (%)	**3.75**	**3.94**	**7.06**	**5.21**	**5.13**	**--**

T_1: Control, T_2: Garlic clove extracts, T_3: Aloe vera leaf extracts, T_4: Black cumin seed extracts, T_5: Neem leaf extracts, T_6: Nativo 75 WG T_7: Provax 200 WP

CHAPTER V

SUMMARY AND CONCLUSION

In Bangladesh, around 80% of people rely on agriculture for their food and livelihood, with wheat being the third most significant crop after rice. Wheat blast, caused by *Magnaporthe oryzae triticum*, is a novel disease in Bangladesh that has resulted in yield losses of up to 100%. Fungicides can injure humans, plants, and beneficial microbes, as well as induce fungicide resistance in pathogens and long-term toxicity in plant components. On the other hand, several botanical pesticides and bio-control agents have been found to be exceedingly harmless and have no detrimental environmental consequences. So, it’s necessary to adopt some new ecofriendly management to control blast disease of wheat in the field.

The experiment was conducted in the field Laboratory, Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207, during the wheat growing season of November 2020 to April 2021 to find out best ecofriendly management for blast disease of wheat.

Among the several environmentaly responsible management options across various phases of wheat growth, neem leaf extract produced the best results in terms of reducing blast disease incidence (%) and severity. With Provax 200 WP, a chemical fungicide that is hazardous to the environment, the lowest blast disease incidence (%) was 7.86, 8.86 and 10.19, during the milking stage, soft dough stage, and hard dough stage of wheat, respectively. With neem leaf extract treatment, a statistically equivalent reduction in blast disease incidence (%) was reported. When there was no chemical or environmentally friendly management, the highest incidence of blast disease (%) recorded was 13.13, 19.73 and 21.12 in the field at all growth stages of wheat.

During the milking stage, soft dough stage, and hard dough stage of wheat, the lowest blast disease severity was 1.03, 1.23 and 1.63, respectively, with Provax 200 WP, a chemical fungicide that is detrimental to the environment. A statistically similar reduction in blast disease severity was found with neem leaf extract application. The highest severity of blast disease at milking stage, soft dough stage and hard dough stage was reported in the field when there was no chemical or ecologically friendly control.

Wheat spikes with the longest length (17.9 cm) were achieved with the Provax 200 WP application for controlling blast disease of wheat. All botanicals showed statistically similar spike length which was statistically different with Provax 200 WP outcomes. The shortest spike length (16.2 cm) was obtained with the control. When provax 200 WP was applied in the field to control wheat blast disease, the highest spikelets panicle^{-1} (21.1) were obtained, which showed statistically equivalent results when neem leaf extract used to control wheat blast disease. Control treatment had the lowest spikelets spike^{-1} (17.1).

Provax 200 WP and neem leaf extract application to control blast disease yielded the highest grain weight per plot of 1379.3 g and 1352 g, respectively. Control treatment yielded the lowest grain weight per plot of 1056.0 g. Provax 200 WP application produced the highest weight of thousands grain (53.4 g) of wheat, which was statistically equivalent to the findings of botanicals application. Lowest grain weight of 48.2 g obtained with control. Provax 200 WP treatment for controlling blast of wheat yielded the highest yield of 2.64 t ha^{-1}, which was statistically equivalent to yield (2.63 t ha^{-1}) achieved with neem leaf extract application. The lowest yield was 2.05 t ha^{-1} when there was no chemical or ecofriendly management for blast disease of wheat.

Regards as the above results, it can be concluded that among all treatments Provax 200 WP and Neem leaf extract performed better compared to other treatments for controlling blast disease of wheat. Provax 200 WP and Neem leaf extract performed best in case of controlling blast disease incidence (%), disease severity at different growth stages of wheat and all other yield attributes of wheat. Provax 200WP is harmful to the environment, but neem leaf extract has no negative effects on the environment. As a result, neem leaf extract is recommended as an environmentally safe management practice for wheat blast disease in different AEZ to test its efficacy before advised to the farmers.

CHAPTER VI

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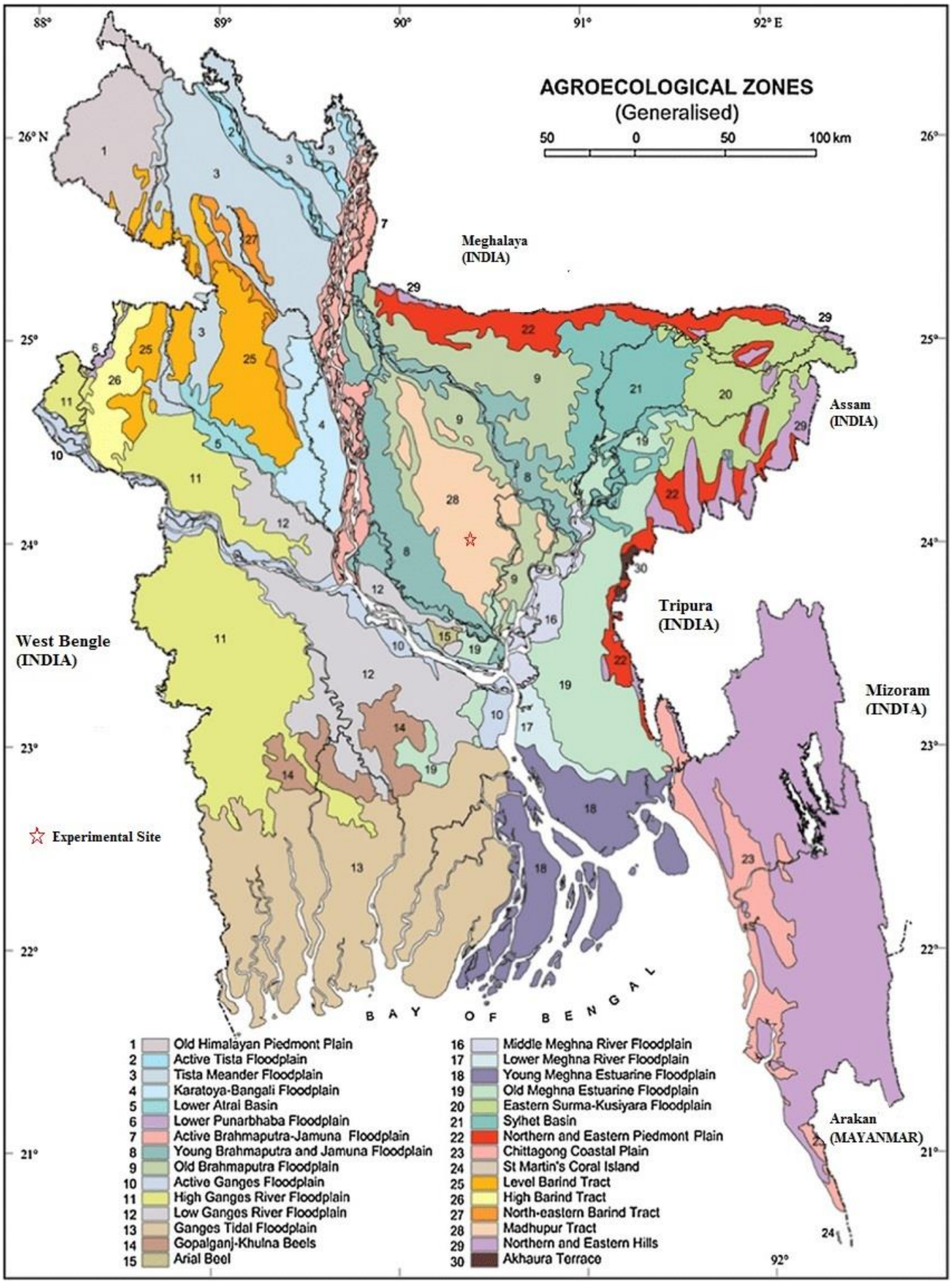
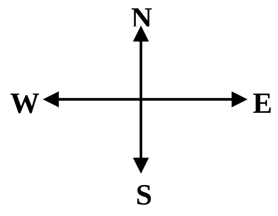
CHAPTER VII

APPENDICES

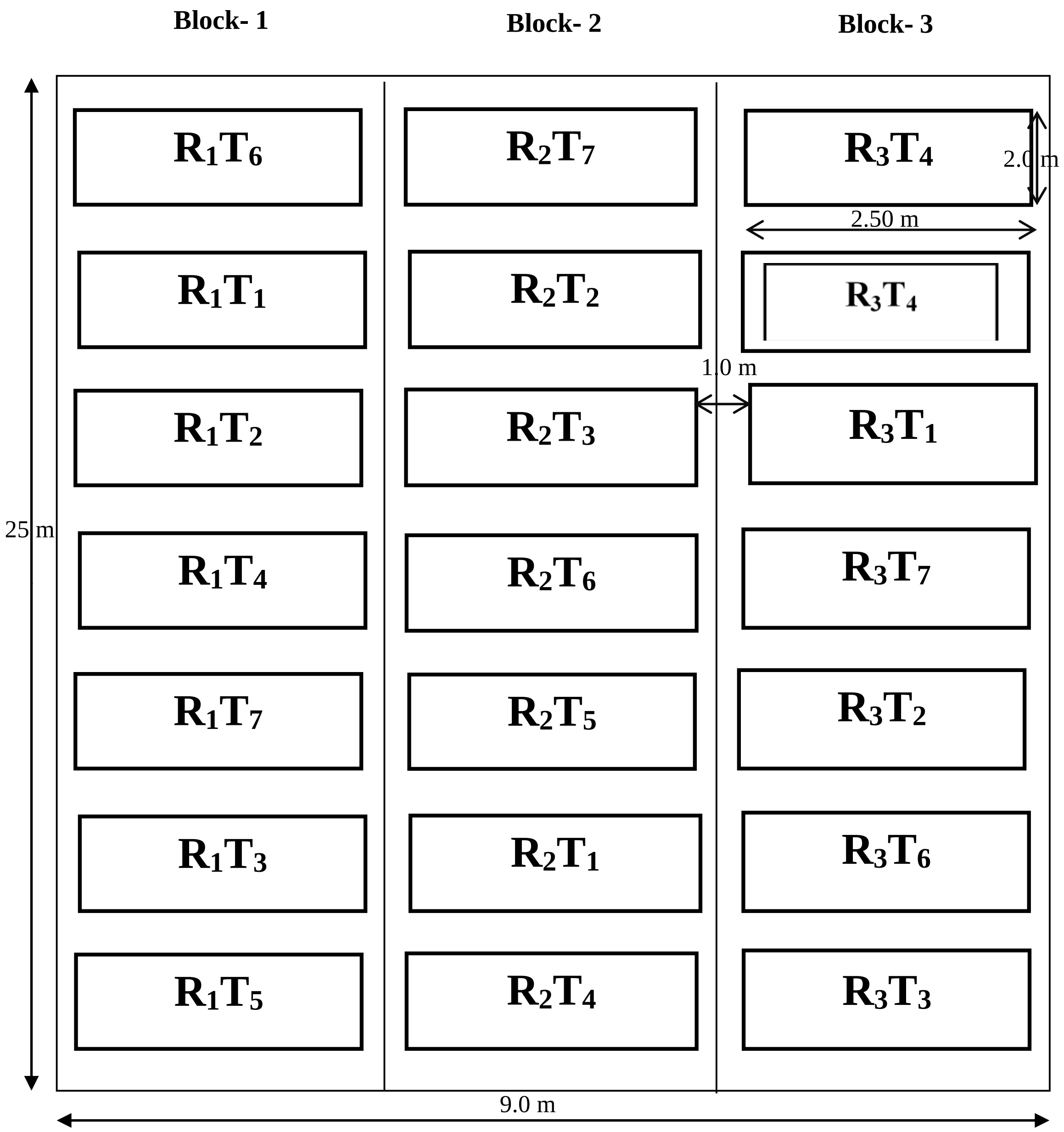
Appendix I: Map showing the location of the site of the experiment.



Appendix II: Field layout of the experiment



Total area	= 25m x 9m
One plot size	= 2.50 m x 2m
Block to block distance	= 1m
Plot to plot distance	= 1 m
Border	= 0.50 m
Total plot	= 21
Design	= Randomized complete block design



Appendix III: Physical and chemical properties of the soil

Characteristics	**Value**
Particle size analysis	
% Sand	30
% Silt	40
% Clay	30
Textural class	Clay loam
Consistency	Granular and friable when dry
pH	5.6
Bulk Density (g/cc)	1.45
Particle Density (g/cc)	2.53
Organic carbon (%)	0.45
Organic matter (%)	0.78
Total N (%)	0.06
Available P (ppm)	20.0
Exchangeable K (meq/100g soil)	0.12

Source: SRDI, 2015

Appendix V: Analysis of variances of the data on different attributes of wheat

Source of variation	Degrees of freedom	Mean Squares					
		Disease Incidence			Disease Severity		
		Milking stage	Soft dough stage	Hard dough stage	Milking stage	Soft dough stage	Hard dough stage
Replication	2	0.4245	8.9297	4.9886	0.00905	0.01286	0.01075
Treatment	6	12.0804	44.7856	57.2991	0.30635	0.39873	0.40552
Error	12	0.7646	1.6947	2.7743	0.00516	0.00897	0.01119

* significant at 0.05 level
** significant at 0.01 level

Appendix IV: Analysis of variances of the data on different attributes of wheat

Source of variation	Degrees of freedom	Mean Squares				
		Grain weight per plot	Weight of thousands grain	Panicle length	Spikelets per panicle	Yield
Replication	2	23957.9	6.93554	0.30143	0.70048	0.02714
Treatment	6	42518.9	8.74375	0.86603	8.70984	0.13056
Error	12	7314.5	6.99959	0.21698	0.32937	0.01476

* significant at 0.05 level
** significant at 0.01 level