DEVELOPMENT OF RESISTANCE SYSTEM IN RICE PLANT AGAINST BLAST DISEASE CAUSED BY *Magnaporthe oryzae* USING SELECTED NOVEL CHEMICALS

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CERTIFICATE

*This is to certify that thesis entitled, "**DEVELOPMENT OF RESISTANCE SYSTEM IN RICE PLANT AGAINST BLAST DISEASE CAUSED BY Magnaporthe oryzae USING SELECTED NOVEL CHEMICALS**" submitted to the Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE (M.S.) in PLANT PATHOLOGY** embodies the result of a piece of bona-fide research work carried out by **ZARIN RAFA URBI** Registration no. **14-05909** 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.

Date: 9 January, 2022
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DEDICATED
TO MY
BELOVED
PARENTS

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ABSTRACT

The present study was conducted in Sher-e-Bangla Agricultural University (SAU) during Boro season 2019-2020.The pot experiment was conducted in net house with CRD design and the field experiment was conducted in central farm of SAU with RCBD design. To conduct the experiments BRRI dhan 28 was used as a cultivar and in total six compounds viz. salicylic acid (T_1), potassium silicate (T_2), silica gel (T_3), P cattle urine (T_4), Peak Performance Nutrient (T_5), N-cattle urine (T_6) were selected to develop induced resistance system in rice plant. From the study, it was revealed that all of the selected novel chemicals showed some extent effectiveness over control to develop the resistant system in rice plant upon artificial inoculation of pathogen in pot and natural condition. Among the selected novel chemicals, cattle urine gave better result to develop the induced resistant system in rice plant upon artificial inoculation as well as in foliar spray. Potassium silicate (@ $4gL^{-1}$) and PPN (@ 8 gm/L) also gave the satisfactory result and plants showed resistance against blast pathogen. Silicon (Si) is known as a “beneficial element” for plants. The direct and indirect benefits of the element for crops (especially grasses) are related to resistance to diseases, pests a drought that again proved from the current study. In this study, yield and yield contributing character like tiller number, panicle mass, plant height (cm) and grain yield (kg) were also studied. Satisfactory results were found in case of treatment cattle urine and potassium silicate. However, it may be concluded that compounds like animal bio-products and silicon containing chemicals can be used to develop resistance system in rice plant against blast pathogen.

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CHAPTER I

INTRODUCTION

Rice is the backbone of Bangladesh's agriculture; here, like in many other countries, 'food security' almost entirely depends on 'rice security' (Brolley, 2015); it alone contributes about 4.5% to the GDP (BBS, 2020). Rice (*Oryza sativa* L.) is the staple food of about 135 million people of Bangladesh. It provides nearly 48% of rural employment, about two-third of total calorie supply and about half of the total protein intake of an average person in the country. Rice sector contributes half of the agricultural GDP and one-sixth of the national income in Bangladesh. Rice (*Oryza sativa* L.) is the source of subsistence for more than one third of human population. In world aspect, rice provides over 21% of the calorific needs of the world's population and fulfils up to 76% of the calorific intake of the population of South East Asia (Fitzgerald *et al*., 2009; Rana *et al*., 2020). It is the main staple food in the Asia and the Pacific region, providing almost 39 % of calories (Yaduraju, 2013). Two types of rice is cultivated world wide Indica and Japonica. These plants are native to Tropical and Subtropical Southern Asia and Southeastern Africa, respectively (Linares, 2002). More than 90% of the world's rice is grown and consumed in Asia where 60% of the earth's people live (Kole, 2006).

It is predicted that the world population will exceed 8 billion people by 2025 and to meet these global food demands, the production of grain needs to increase up to 50% more by the year 2025 (FAO, 2012). Rice grain contains on an average 7% protein, 62-65 % starch, 0.7% fat and 1.3% fiber and rice is a main source of vitamin B1 (thiamin), B2 (riboflavin), B3 (niacin) and B5 (pantothenic acid). The Biological value of rice is 63% whereas Biological value of wheat and maize is 49% and 36%, respectively. Rice is a rich source of carbohydrates, proteins and vitamins providing 84 to 310 kilo calories of energy. Nutritionally, hundred grams of rice provides 6.9 g of protein, 1.3 g of dietary fiber, 0.2 g of sugar, 3.7 g of carbohydrates, 0.008 g calcium, 0.23 g of iron, 0.049 mg of riboflavin, 0.10 mg of thiamine, 0.018 mg of pentothenic acid, 1.3 g of nicotinic acid, 0.158 mg of vitamin B6 and also 1.05 mg of manganese, 115 g of phosphorus, 25 mg of magnesium, 115 mg of potassium, 1.09 mg of zinc and 0.01 mg of vitamin C (Swaminathan, 1995).

Rice crop is subjected to attack by 50 diseases include 21 fungal, 6 bacterial, 4 nematodes, 12 viral and 7 miscellaneous diseases and disorders (Jabeen *et al*., 2012). Among the major rice diseases that often cause great economic losses, rice blast is a vicious threat to the country's economy (Ganesh *et al*., 2012). Outbreaks of rice blast are a serious and recurrent problem in all rice growing regions of the world. It is estimated that each of the three years enough of rice is destroyed by rice blast alone to feed 60 million people (Zeigler, Leong, &Teng, 1994).Out of the total yield loss due to diseases in rice, 35% is by blast, 25% by sheath blight, 20% by BLB, 10% by tungro and remaining 10% by other diseases. The disease causes yield losses from between 1-100% in Japan (Kato, 2001), 70% in China, 21-37% in Bali Indonesia (Suprapta and Khalimi, 2012), and 30-50% in South America and Southeast Asia (Baker *et al*., 1997).

Rice blast is the most important fungal rice disease, which is caused by the fungus *Magnaporthe oryzae*. Rice blast, caused by *Magnaporthe oryzae* is ranked first among the most destructive diseases of rice, causing yield reduction by 10–30%, even 100% in severe cases (Dean *et al*., 2012; Li *et al*., 2019; Liu *et al*., 2014). This disease has been reported to occur in more than 85 countries. It is estimated that 60 million people could feed each year by the produce of rice which is destroyed by rice blast (Zeigler *et al*., 1994). Rice blast was first reported in China by 1637, in Japan in 1704, and in the United States and India in 1876 and 1913, respectively (Ou, 1971).

Finally rice blast can infect rice plant right from seedlings to adult plant stages affecting leaves, nodes, collar, panicles and roots. Though chemical fungicides are able to control plant diseases effectively, but the excessive use of chemicals leads to serious concerns for the environment and causes human health problems. Chemical residues in soil make it infertile, and disturb the proper growth and development of plants. In considering the above mentioned circumstances, the prime aim of the present study was to evaluate the selected novel chemicals for development the induced resistance system in rice plant and to determine the effects of noble chemicals against rice blast to minimize the use of fungicides and mitigate the environmental pollution.

Objectives:

-To evaluate the efficacy of selected novel chemicals to develop resistance system in rice plant against rice blast.

-To determine the efficacy of selected novel chemicals in pot and field conditions against rice blast pathogen.

CHAPTER II

REVIEW OF LITERATURE

The available literatures on blast management have been reviewed in this chapter. The review of literatures related to the study is presented using various headings and sub-headings.

Importance of Rice

Rice, a staple food for more than half of the world's population, is grown in >100 countries with 90% of the total global production from Asia. Rice, wheat, and maize are the world's three leading food crops; together they directly supply more than 42% of all calories consumed by the entire human population. Human consumption in 2009 accounted for 78% of total production for rice, compared with 64% for wheat and 14% for maize. Of these three major crops, rice is by far the most important food crop for people in low- and lower-middle-income countries. Rice is the staple food of more than half of the world's population – more than 3.5 billion people depend on rice for more than 20% of their daily calories. Rice provided 19% of global human per capita energy and 13% of per capita protein in 2009.

Asia accounts for 90% of global rice consumption, and total rice demand there continues to rise. But outside Asia, where rice is not a staple yet, per capita consumption continues to grow. Rice is the fastest growing food staple in Africa, and also one of the fastest in Latin America. In Asia**,** rice consumption is very high, exceeding 100 kg per capita annually in many countries. For about 520 million people in Asia, most of them poor or very poor, rice provides more than 50% of the caloric supply (Rice pedia).Rice provides more than 15 essential vitamins and minerals, including folic acid, B vitamins, potassium, magnesium, selenium, fiber, iron and zinc.

Rice provides 20% of the world's dietary energy supply, while wheat supplies 19% and maize 5%. Rice grain contains on an average 7% protein, 62-65 % starch, 1.3% fibre and0.7% fat. It is also a main source of vitamin B1 (thiamin), B2 (riboflavin), B3 (niacin) and B5 (pantothenic acid).The Biological value of rice is 63% whereas Biological value of wheat and maize is 49% & 36% respectively. In Bangladesh more than 95% of population consumes rice and it alone provides 76% of calorie and 66% of total protein requirement of daily food intake (Bhuiyan et al., 2002). Thus, rice plays a vital role in the livelihood of the people of Bangladesh.

Importance of blast disease of rice

Fungal diseases alone are estimated to reduce annual rice production by 14% globally (Agrios, G.N,2005).Rice blast disease, caused by *Magnaporthe oryzae* , occurs in about 80 countries on all continents where rice is grown, in both rice fields and upland cultivation. The extent of damage caused depends on environmental factors, but worldwide it is one of the most devastating cereal diseases, resulting in losses of 10–30% of the global yield of rice. In rice seedlings, small necrotic regions appear initially, which become larger and coalesce, and have chlorotic margins. In older rice plants, disease symptoms can occur in leaves, collar – junction of the leaf blade and leaf shethe, nodes, neck, and panicle. Neck rot and panicle blast are particularly devastating causing up to 80% yield losses in severe epidemics (Lynne Boddy, 2016). Rice blast (*Magnaporthe oryzae*) is a key concern in combating global food insecurity given the disease is responsible for approximately 30% of rice production losses globally—the equivalent of feeding 60 million people (Nawton Nalley, 2016). Yield loss from blast infections depends on varietal susceptibility, the degree of infection, and the timing of fungicide application. Some yield losses associated with blast outbreaks have reached 50% or more (Khush GS, 2009)

Symptoms of the blast disease

The rice blast pathogen infects all the aerial parts of the rice plant at various growth and developmental phases like leaf, leaf sheath, internodes, nodes, internodes, neck, and panicle (Castilla N, 2009). The rice blast pathogen develops in the nodes, leaves, collars, necks, panicles, seeds and roots over the entire growth period (Sesma, A., 2004). The severity of infection depends on the environmental conditions prevailing the area, the age of the host plant and the degree of resistance of the rice plant. The leaves are the most affected plant part by the pathogen. The fungus subsequently invades the above-ground parts of the rice plant and, in severe epidemics large ellipsoid lesions can engulf the entire surface of a leaf (Talbot, N.J, 1995). The foliar lesions reduce the leaf area that should be available for photosynthesis thereby reduces grain yield in return. Severe infection at the early stage of the plant tiller may destroy it. Whenever the pathogen attacks the neck and node of the rice plant, the plant tissues will be disorganized and this inhibits the movement of water and nutrient that ensure grain filling for good yield. The disease initially appears as whitish or grayish specks along the leaf margins. Later on they turn into elliptical spots which are elongated and diamond shaped with pointed ends. These spots become necrotic in the center with brown or reddish-brown margins. These spots collapse each other and forms large lesions. On stem, fungus produced elongated, grayish to black color lesions. Disease also appears on rice seeds as brown diamond shaped spot (Hajano et al. 2011). Neck and node blast result to early panicles maturity that brings about yield losses through grain shedding and the quality of the harvested grain is reduced (Zhu YY, 2005).Early neck infection of the plant brings about inhibition of grain filling while partial grain filling will occur in late infection (Padmanabhan SY, 1974).Partial to complete sterility may occur when the last node is severely infected (Ram T, 2007). Node and panicle blast have been described as the greatest destructive disease of rice at the reproductive and ripening phase (Bonman JM, 1991). The infected panicle usually gets broken and falls off; even the inflorescence may break off as a result of rotten node that can no longer support it again. Seeds fail to develop when the pedicles become infected, a condition called seed blanking. Based on the rice blast attack mechanism, it has been found that the damage is very much influenced by environmental factors. The disease can survive throughout the year in the air and can be severed during periods of low temperatures and high moisture; while conidia do not germinate under direct sunlight; overcast conditions and dew encourage blast spread (Ou, S.H, 1985).Blast lesions occur in suitable weather, leading to increased blast incidence and severity for 7 to 10 days then destroying plants within 15 to 20 days, causing yield losses of up to 100% (Musiime, O., 2005).

Morphology of the blast pathogen

The fungus conidia size is 20-22 × 10-12 μm which are translucent, two-septate, and slightly darkened. The growth of mycelia, conidia formation and conidial germination of the conidial of the pathogen can occur at all pH level for except2.35-2.95 with optimal conditions for mycelia growth, formation of conidia and germination of conidia were maximum at the pH of 4-6, 4.60-6.45 and 4.60 – 5.45 respectively. The fungus conidia size is 20-22 × 10-12 μm which are translucent, two-septate, and slightly darkened. The growth of mycelia, conidia formation and conidial germination of the conidial of the pathogen can occur at all pH level for except2.35-2.95 with optimal conditions for mycelia growth, formation of conidia and germination of conidia were maximum at the pH of 4-6, 4.60-6.45 and 4.60 – 5.45 respectively. The fungus conidia size is 20-22 × 10-12 μm which are translucent, two-septate, and slightly darkened. The growth of mycelia, conidia formation and conidial germination of the conidial of the pathogen can occur at all pH level for except 2.35-2.95 with optimal conditions for mycelia growth, formation of conidia and germination of conidia were maximum at the pH of 4-6, 4.60-6.45 and 4.60 – 5.45 respectively. This pathogen, *Magnaporthe oryzae* is filamentous ascomycetes in nature with the capability to produce sexually and asexually. The fungus conidia size is 20-22 × 10-12 μm which are translucent, two-septate, and slightly darkened. The growth of mycelia, conidia formation and conidial germination of the conidial of the pathogen can occur at all pH level for except 2.35-2.95 with optimal conditions for mycelial growth, formation of conidia and germination of conidia were maximum at the pH of 4-6, 4.60- 6.45 and 4.60 – 5.45 respectively

Disease cycle of *Magnaporthe oryzae*

Conidiophores produced from the autophagic cell death of conidia are transmitted to other plant tissues or nearby by plants by wind, working tools, water splash or plant contact start a new infection cycle (Veneault-Fourrey C, 2006).The pathogen conidia can spread within 230 meters from its source when the environment is favorable; high relative humidity with winds of 3.5 m s-1 or more (Kingsolver CH, 1984). Airborne Magnaporthe oryzae conidia exist all year-round and are responsible for epidemics occurrence throughout the year (Guerber C, 2006). Longer period of leaves dampness, relative humidity of about 92-96% and the air temperature around 25-28°C were environmental factors that favor spores growth and lesion development (Kankanala P,2007). However, reports from several researches have indicated that a high dosage of nitrogen supply favors heavy *Magnaporthe oryzae* infection.

Selected novel chemicals against blast pathogen

Silicon (Si), a beneficial element for plant growth, has shown to provide a prophylactic effect against many pathogens. The application of Si helps the plants to combat the disease-causing pathogens, either through its deposition in different parts of the plant or through modulation/induction of specific defense genes by yet an unknown mechanism. Some reports have shown that Si imparts resistance to rice blast and sheath blight (Atul Prasad Sathe, 2021). Silicon has long been known for its beneficial effects on plant growth, especially under stress conditions (Epstein, 2009; Frew *et al*., 2018). Silicon alleviates stress conditions imposed by biotic factors like pathogens and pests and abiotic stresses such as drought, salt, and metal toxicities (Ma, 2004). Considering the convincing reports suggesting the beneficial effect of Si particularly in stress condition, Si based fertilizers are being included in agronomical practices in rice (Devanna *et al.*, 2021). Significant yield increases with Si fertilizer have been reported in rice even without imposing any stress (Pati *et al*., 2016; Devanna *et al.*, 2021).

Considering the beneficial effects off the chemical we have tried to identify some affordable products which can be used as novel chemicals.

CHAPTER III

MATERIALS AND METHOD:

The present study having the title "**Development of Resistance System In Rice Plant Against Blast Disease Caused By *Magnaporthe Oryzae* Using Selected novel chemicals**" was carried out in the Department of Plant Pathology, Sher-e-Bangla Agricultural University, Dhaka-1207. The detailed information regarding the materials used and methodology followed during the study are described below with some headings and sub-headings.

3.1. Experimental site

The experiment was conducted in two steps. For pot experiment was conducted in the net house under the Department of Plant Pathology. And the field experiment was conducted at central farm of Sher-e-Bangla Agricultural University, Dhaka -1207. The experimental field area was situated at 23°46' N latitude and 90°22' E longitude at an altitude of 8.6 meter above the sea level (Anon. 1988).

3.2. Experimental duration

The pot experiment was conducted during November, 2019-May, 2020 and the field experiment during November, 2020-May, 2021.

3.3. Experimental materials collection

3.3.1: Variety selection and Seed collection

BRRI Dhan-28 was selected due to its susceptibility to rice blast pathogen & wide spread cultivation during Boro season in Bangladesh. Seeds were collected from Bangladesh Agricultural Development Corporation (BADC).

3.3.2: Pure culture collection

Pure culture of the ***Magnaporthe oryzae*** pathogen was collected from plant pathology lab of Bangladesh Rice Research Institute (BRRI).

Figure 1: Collected pure culture preserved in a test tube

3.4. Pot Experiment

3.4.1 Sprouting of seeds

Seeds were soaked in plastic pots with tap water overnight. Before sowing in seed bed seeds were taken out from water, put in gunny bags and kept at room temperature for 72 hours for sprouting.

Figure 2: Sprouted seeds after 72 hours

3.4.2 Seed bed preparation and sowing of sprouted seeds

Seed bed was prepared at central farm, Sher-e-Bangla Agricultural University, Dhaka. Power tiller & harrow were used to do so and manuring was done as required. Sprouted seeds were sown in wet seedbed. Weeding and irrigation was done in the seed bed whenever needed.

Figure 3: Seedlings grown in seedbed

3.4.3. Pot preparation and seedling transplantation

For pot experiment, pots were collected from nearby market and kept in net house. Fertilizers were applied to the pot soil before transplantation. The doses were cow dung (250 g /pot), Urea (50 g /pot), TSP (50 g /pot), MP (50 g /pot), Gypsum (50 g /pot), Boron (10 g /pot), Zinc sulphate (10 g / pot), then mixed them with soil properly. 35 days old rice seedlings (BRRI dhan 28) was transplanted in the prepared pots.

a b c

Figure 4: Preparation of pots by mixing soil and fertilizers accordingly; a. measured fertilizers for each pot, b. prepared pots, c. sowing of seedlings

3.4.4. Intercultural operations

Intercultural operations were maintained from transplantation to mature stage. Weeding, mulching, thinning, gap filling, earthing up etc. were done where and when necessary.

3.4.5. Selected novel chemicals added to the soil and applied as foliar spray

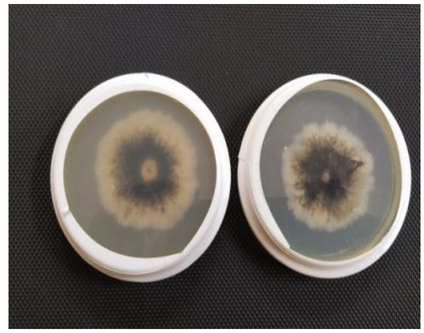
In total six (6) compounds were selected to conduct the pot experiment with three replications in CRD design. **S**ilica gel was added to the soil, potassium silicate (K_2SiO_3) and salicylic acid was sprayed @ $4gL^{-1}$; cattle urine (normal cow) and cattle urine (pregnant cow) were sprayed freshly; peak performance nutrient (PPN) were sprayed @8 $g\ L^{-1}$ .Chemicals were sprayed 3 times, initially at 35 DAT. Then second and third sprays were done before panicle initiation and heading stage respectively.

Figure 5: Spraying of selected novel chemicals in different doses

3.4.6. Re-culture of *Magnaporthe oryzae*

The experiment was carried out in Molecular Biology and Plant Virology Lab under the Department of Plant Pathology, SAU.

Petri dishes with PSA media were prepared. A portion of mycelium was transfer to PSA media and placed in the incubation chamber for 15-20 days at 28 $\pm 1^0$C. Then a portion of culture was taken on slide and observed under microscope to study the pathogenic structure of ***Magnaporthe Oryzae*** with the help of relevant literature (Mew and Gonzales, 2002; Ellis, 2001; Barnet and Hunter, 1972).



a **b**

Figure 6: *Magnaporthe oryzae* pathogen was recultured; a. pure culture of blast pathogen, b. recultured mycelia growth of the fungus

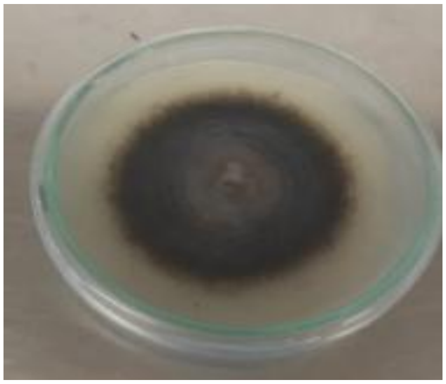
Preparation of Potato Sucrose Agar (PSA) Media: PSA media was prepared according to standard protocol. The composition of PSA media is given below:

Ingredient	Amount
Potato infusion	200 gm
Sucrose	20 gm
Agar	17 gm
Distilled Water	1 liter

Firstly, 200 gm sliced potatoes were boiled in 1 liter water. Then potato infusion was collected by filtration. Then sucrose and agar were added proportionately. Then the media was autoclaved at 121℃ for 15 minutes. Then they were transferred to petridishes aseptically.

Cultural, Morphological and Sporulation study of *Magnaporthe oryzae:*

Magnaporthe was identified based on their morphological growth pattern on PSA medium. Morphological variation was examined and characterized according to their conidial structure (size, shape, color and septation of conidia). Mycelial growth, conidiophore and conidia were observed under light microscope. Sporulation study was done using hemo-cytometer.



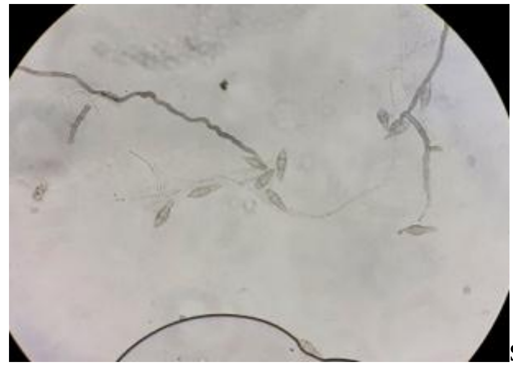

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Figure 7: Morphological view of the pathogen under light microscope

3.4.7. Inoculation of Pathogen

Spore suspension (1x10^^{5} spores/ml) ***Magnaporthe oryzae*** was artificially inoculated to the pot plants @50 DAT. Proper safety measures and inoculation principles were followed. The whole process was done under controlled condition.

3.4.8. Assessment of Disease Incidence and Severity

Disease incidence and severity were evaluated after 15 days of inoculation.

Disease incidence was calculated by the following formula (Greer &Webster, 2001):

$$\text{Disease Incidence (\%)} = \frac{\text{Number of diseased tillers}}{\text{Total number of inspected tillers}} \times 100$$

Disease severity of was recorded by SES IRRI, 1996 used a 0-9 scale for predominant lesion type.

0= No lesion observed= Highly resistant =0%

1= 10% leaf area covered by blast spots= Resistant = 10%

2-3=20-30% leaf area covered by blast spots = Moderately resistant = 20-30%

4-5= 40-50% leaf area covered by blast spots =Moderately susceptible= 40-50%

6-7 =60-70% leaf area covered by blast spots = Susceptible= 60-70%

8-9=80-90% leaf area covered by blast spots = Highly susceptible = 80-90%

3.5. Field Experiment

3.5.1 Seed collection

BRRI Dhan-28 was selected due to its susceptibility to rice blast pathogen & wide spread cultivation during Boro season in Bangladesh. Seeds were collected from Bangladesh Agricultural Development Corporation (BADC).

3.5.2. Land preparation

The land was prepared with the help of power tiller and harrow. The land was first opened on January 2021 and ploughed. The final ploughing was done with the help of power tiller followed by laddering in order to level the soil surface. Weeds and stubbles were removed from the land.

Figure 8: Preparation of land for transplantation

3.5.2. Fertilizer application

Fertilizers were applied as per recommendation of BARC, 2012 (Fertilizer Recommendation Guide). All fertilizers except 2/3rd urea were incorporated with soil during final land preparation. Rest of the urea was applied at 30 and 45 DAT in equal two installments. The following doses of fertilizers were applied to the plots:

Fertilizer	Dose (kg/ha)
Urea	100
TSP	16
MP	66
Gypsum	12
Zinc Sulphate	1.3

3.5.3. Design and layout

The experiment was laid out in randomized complete block design (RCBD) with three replications. Blocks represented the replication. Each block comprised eight unit plots and total number of plots were 24 (7 X 3=21). Size of each unit plot was 3.0 X 2.28 = 6.84 m^2.The distances between unit plots were 1.0 m and blocks 1.5 m.

3.5.4. Seedling transplantation

Seedlings were uprooted from the bed very carefully, and then transplantation was done on January, 2021 in the main field. Row to row spacing was maintained as 1m and hill to hill 20 cm. Three seedlings were transplanted together in an individual hill.

3.5.5. Intercultural operation

Weeding, irrigation, gap filling, fertilizer and insecticide application was done in the field whenever necessary**.**

3.5.6. Assessment of the disease incidence and severity in the field

Each of the plots was investigated for recording the incidence and severity of Blast disease. Visual symptoms were observed for to record data. Affected plants from each unit plot were selected for assessing the incidence and severity. Data were recorded three times at an interval of 20 days (45 DAT, 65 DAT, and 85 DAT). Data were recorded on; hill/plot, infected hill/plot, total leaf/infected hill, infected leaf/infected hill, % disease infection/leaf. To estimate the incidence of Blast disease in grain five hill of each unit plot were 12 harvested randomly and separately during ripening stage. Then fifteen hill (As each variety consists three replication) from each variety were mixed together and select 30 panicle randomly from that mixture. Data were recorded on grain/panicle and infected grain/panicle.

Figure 8: Spraying of novel chemicals in field

Disease Incidence: % disease incidence was estimated by using the following formula (Rajput and Bartaria, 1995)

$$\% \text{ disease incidence} = \frac{\text{No. of infected hill or hill parts}}{\text{No. of inspected hill or hill parts}} \times 100$$

Disease Severity: The severity of the disease was recorded by IRRI recommended grading scale (0-5 scale of Standard Evaluation System for Rice, 1980).It was firstly used by American Scientist Dr. Horsfall and Barrett (1945).

The grades of the disease scale are given below:

%Leaf Area Disease (%LAD)	Rating/Grading
0	0
0.1-5.0	1
5.1-12.0	2
12.1-25.0	3
25.1-50.0	4
>50.0	5

3.5.8 Data Analysis:

Statistix-10 statistical software was used to analyze the data. The mean data was taken @ 5% level of significance

CHAPTER IV

RESULTS

4.1. Pot Experiment

4.1.1 Effect of selected chemicals on inducing disease resistance in rice varieties under pot conditions

Data on disease incidence represents that the highest disease incidence range (55.77-90%) was estimated in control treatment whereas the lowest disease incidence range (0.00-3.5%) was recorded in T4 treatment (P cattle urine) in all of the observations. Data on disease severity range varied from 0-75% at the same dose of chemicals at different observations. The highest disease severity range (33.33-75%) was recorded in control pot and the lowest disease severity range (0.00-4.50%) was found in treatment, T4 (P cattle urine) at different data collection date. Among the selected chemicals; cattle urine, PPN and potassium silicate (K2SiO3) gave the best results to develop the induced resistant system in rice plant (BRRI dhan 28) and prevent the infection of blast pathogen. These results are presented in table 1 and 2.

Table 1. Influence of selected chemicals in development of induced resistance system and *Magnaporthe oryzae* inoculation on disease incidence (%) in boro rice variety (BRRI dhan 28)

Treatment	Disease incidence (%) @ 65 DAS	Disease incidence (%) @ 80 DAS	Disease incidence (%) @ 95 DAS	Disease incidence (%) at panicle initiation stage
Control (T_0)	55.77 a	55.77 a	63.39 a	90 a
SA (T_1)	5.71 b	8.01 b	12.06 b	40 b
K-silicate (T_2)	3.17 b	3.17 c	3.17 c	20 c
Silica gel (T_3)	6.52 b	7.76 b	7.75 b	40 b
Pregnant cattle urine (T_4)	0.00 c	1.35 c	3.40 c	3.5 e
PPN (T_5)	1.15 b	3.45 c	4.56 b	5.5 e
Normal cattle urine (T_6)	2.47 b	3.90 c	5.75 b	10 d
CV (%)	15.21	15.53	13.58	17.38

Table.2. Influence of selected chemicals in development of induced resistance system and *Magnaporthe oryzae* inoculation on disease severity (%) in boro rice variety (BRRI dhan 28)

Treatment	Disease severity (%) @ 65 DAS	Disease severity (%) @ 80 DAS	Disease severity (%) @ 95 DAS	Disease severity (%) at panicle initiation stage
Control (T_0)	33.33 a	33.33 a	46.67 a	75 a
SA ($T_{1)}$	10.0 b	13.33 ab	16.67 c	53 c
K-silicate (T_2)	6.67 b	8.33 b	10.0 e	42 d
Silica gel (T_3)	10.0 b	15.00 ab	33.33 b	62 b
Pregnant cattle urine (T_4)	00.0 c	1.50 c	3.0 f	4.5 f
PPN (T_5)	3.33 b	3.33 c	5.0 f	6.0 e
Normal cattle urine (T_6)	10.0 b	10.0 ab	13.33 d	11 e
CV (%)	10.0	11.47	14. 37	15.89

4.1.2 Effect of selected chemicals on tiller number and panicle mass in pot condition

A comparison of tiller number and panicle mass per pot indicated (Table 3) that there were significant effects of the chemicals. The tiller number range was 9.67-27.66 .The seedlings treated with urine of pregnant cow (treatment T4) had the highest tiller number which was 27.66. The untreated seedlings had the lowest tiller numbers which was 9.67.For panicle mass/pot the range was 8.67-25.7.So, again the treatment T4 showed best performance by having the highest panicle mass which was 25.7g. The lowest panicle mass was again found in control treatment which was 8.67.

Table.3. Effect of selected chemicals applied in soil and foliar spraying on number of tiller/pot and panicle mass/pot

Treatment	Number of tiller/pot	Panicle mass/pot
Control (T_0)	9.67 c	8.67 c
SA ($T_{1)}$	13.33 b	11.33 b
K-silicate (T_2)	23.00 ab	21.33 ab
Silica gel (T_3)	15.00 b	13.0 b
Pregnant cattle urine (T_4)	27.66 a	25.7 a
PPN (T_5)	23.3 ab	20.33 b
Normal cattle urine (T_6)	22.0 ab	20.0 ab
CV (%)	3.78	3.21

4.2. Field experiment

4.2.1 Effect of chemicals on inducing disease resistance in rice varieties under field conditions

Data on disease incidence represents that the highest disease incidence (5.8) was found in T3 among the selected chemicals. The lowest disease incidence range 3.21% was recorded in T4 treatment (P cattle urine). It is also seen that the novel chemicals have statistical non-significance. Overall the highest disease incidence (11.20%) was recorded in control
treatment.

Table.4. Influence of selected chemicals in development of induced resistance system and *Magnaporthe oryzae* inoculation on disease incidence (%) in boro rice variety (BRRI dhan 28) in field condition

Treatment	Disease incidence (%)
Control (T_0)	11.20 a
SA ($T_{1)}$	4.87 b
K-silicate (T_2)	4.56 b
Silica gel (T_3)	5.80 b
Pregnant cattle urine (T_4)	3.21 b
PPN (T_5)	3.90 b
Normal cattle urine (T_6)	4.23 b
CV (%)	3.33

4.2.2 Effect of noble chemicals on tiller number and panicle mass in field condition

A comparison of tiller number and panicle mass per plot indicated (Table 2) that there were significant effects of selected chemicals. The tiller number range was 9.73-23.40.The seedlings treated with urine of pregnant cow (treatment T4) had the highest tiller number which was 23.40. The untreated seedlings had the lowest tiller numbers which was 9.73.For panicle mass/plot the range was 5.33-28.30.So, again the treatment T4 showed best performance by having the highest panicle mass which was 28.30g. The lowest panicle mass was again found in control treatment which was 5.33.

Table.5. Effect of selected chemicals applied in soil and foliar spraying on number of Tiller/hill and panicle mass/hill

Treatment	Number of tiller/hill	Panicle mass/hill
Control (T0)	9.73 d	7.33 c
SA (T1)	16.33 bc	15.30 b
K-silicate (T2)	21.20 ab	11.91 c
Silica gel (T3)	13.60 cd	10.20 c
Pregnant cattle urine (T_4)	23.40 a	20.30 a
PPN (T5)	19.60 ab	17.40 b
Normal cattle urine (T_6)	20.27 ab	13.52 c
CV (%)	5.6113	2.5887

4.2.3 Effect of selected chemicals on plant height and panicle length in pot condition

Significant effects of selected chemicals were recorded on plant height and panicle length (indicated in Table 3). The plant height range was 68.27-82.40 .The seedlings treated with urine of pregnant cow (treatment T4) had the highest tiller number which was 82.40. The untreated seedlings had the lowest tiller numbers which was 68.27.For panicle length the range was 11.07-24.32.So, again the treatment T4 showed best performance by having the highest panicle mass which was 24.32g. The lowest panicle mass was again found in control treatment which was 11.07.

Table.6. Effect of selected chemicals applied in soil and foliar spraying on Panicle length

Treatment)	Plant height	Panicle length
Control (T_0)	68.27 b	11.07 e
SA ($T_{1)}$	77.13 a	17.91 c
K-silicate (T_2)	75.54 ab	20.78 b
Silica gel (T_3)	75.15 ab	16.21 d
Pregnant cattle urine (T_4)	82.40 a	24.32 a
PPN (T_5)	78.20 a	20.80 b
Normal cattle urine (T_6)	77.18 a	20.58 b
CV (%)	8.37	1.092

Significant effects of selected chemicals were recorded on plant height and panicle length (indicated in Table 3). The plant height range was 68.27-82.40 .The seedlings treated with urine of pregnant cow (treatment T4) had the highest tiller number which was 82.40. The untreated seedlings had the lowest tiller numbers which was 68.27.For panicle length the range was 11.07-24.32.So, again the treatment T4 showed best performance by having the highest panicle mass which was 24.32g. The lowest panicle mass was again found in control treatment which was 11.07.

4.2.3 Effect of selected chemicals on yield in field condition

Data on yield represents that the highest yield was recorded in case of T4 treatment (P cattle urine) which was 5.04 kg/plot .The lowest yield recorded in case of control treatment which was 3.52 kg/plot. So, cattle urine had the best results in all of the observations. It is also seen here that the treatments had statistical non-significance.

Table.7. Effect of selected chemicals applied in soil and foliar spraying on yield in field condition

Treatment	Yield Kg/Plot
Control (T_0)	3.52 c
SA ($T_{1)}$	4.29 abc
K-silicate (T_2)	4.76 ab
Silica gel (T_3)	4.25 abc
Pregnant cattle urine (T_4)	5.04 a
PPN (T_5)	4.13 bc
Normal cattle urine (T_6)	4.42 abc
CV (%)	1.26

4.3 Lab experiment

4.3.1 Isolation of *Magnaporthe oryzae*

Isolation of fungi was done by tissue planting method (Agrios, 2006).Firstly, rice diseased rice leaves were collected from the experiment field. Then leaves were cut into pieces and placed on petri dishes with sterile blotter paper. The petri plates were labeled and placed in the incubation chamber for 7 days at 28 $\pm 1^0$C. During incubation they were exposed to florescence light for 12 hours per day. After incubation period, the fungus was grown on the petri plates containing sterile blotter paper. A portion of mycelium was transfer to PSA media and placed in the incubation chamber for 15-20 days at 28 $\pm 1^0$C. A portion of culture was taken on slide and observed under microscope and identified the pathogenic fungi that is *Magnaporthe oryzae* with the help of relevant literature (Mew and Gonzales, 2002; Ellis, 2001; Barnet and Hunter, 1972).

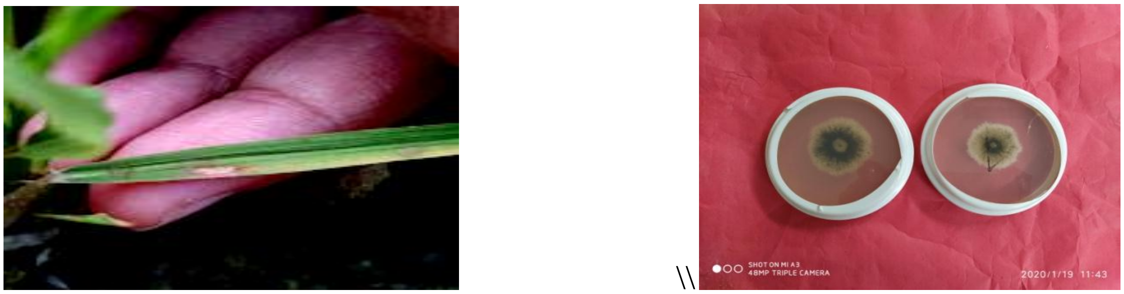

Figure .9: Diseased leaves of rice collected from field experiment and pathogenic growth of *Magnaporthe* PSA media

Cultural, Morphological and Sporulation study of *Magnaporthe oryzae*

The fungus was identified based on its growth pattern. Morphological features such as size, shape, color and conidial septation were used to examine and characterize the fungus.).

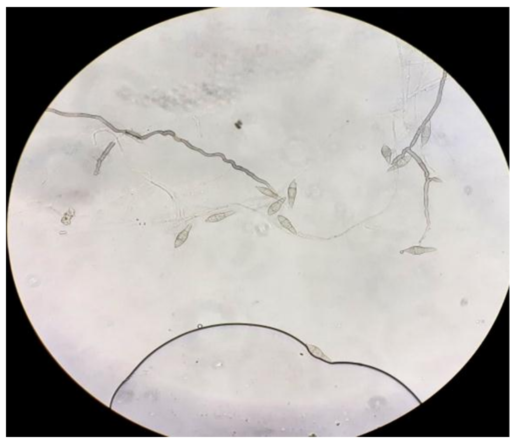

Figure .10: Conidia of *Magnaporthe oryzae* observed under light microscope

Mycelial growth, conidiophore and conidia was observer under light microscope, at first low power and then with high power objective lens. Sporulation study was done by using hemo-cytometer.

CHAPTER V
DISCUSSION

Rice (*Oryza sativa* L) is a dietary staple foods and one of the most important cereal crops, especially for people in Asia, but the consumption outside Asia has increased, recently (Orthoefer, 2005). Rice is also one of food which is considered to be a potential food vehicle for the fortification of micronutrients because of it's regularly consumption. A study in Bangladeshi children and their care givers showed that rice was the main source of zinc intake, providing 49% of dietary zinc to children and 69% to women (Arsenault et al., 2010). The nutrients content of rice were varies depending on the variety of rice soil, and the conditions they growth. Rice contributes to the major dietary energy for body. Pre-germinated brown rice has protein two times more than white rice, i.e. 14.6 g/100 g (brown rice) vs 7.3 g/100 g (white rice). On the other hand, the fat content is so high, namely 24.8 g/100 g for pre-germinated brown rice and 1.5 g/100 g for white rice (Seki et al., 2005). Rice is fortified with some minerals such as iron (Fe) and zinc (Zn), to prevent diseases associated with mineral deficiencies (Sperottoa et al., 2012). As per the USDA data, with a production of 3.6 crore tonnes of rice Bangladesh stands in third position globally in rice production after China and India that produce 14.6 crore tonnes and 11.6 crore tonnes respectively. Currently, as much as 78% of Bangladesh's total arable land is occupied by rice cultivation, resulting in very little land left for growing other crops and thereby Bangladesh remains import-dependent for varieties of other crops like wheat, maize, oilseeds and spices. embarking on a multipronged approach of genetic gains, area expansion and better crop management the government wants to increase rice yield from over 38 million tons now to 47 million tons by 2030 and 54 million tons by 2040 and over 60 million tons by 2050. Considering the above facts and biotic stresses in rice production in Bangladesh, the present study was undertaken to manage the one major disease blast which showed severe outbreak in last few couple of years. In this study several novel chemicals were tested to develop the induced resistance system in rice plant. They were tested both in pot and field condition to measure their effectiveness. All of the selected novel chemicals *viz.* salicylic acid, potassium silicate, silica gel, cattle urine and PPN were showed some extent effectiveness over control to develop the resistant system in rice plant upon artificial inoculation of pathogen and in natural condition. Among the selected novel chemicals, cattle urine gave better result in terms of percent disease incidence and severity. This result agreed with previous report, Akhilesh *et al.* (2017) examined several animal products against blast pathogen and found different degrees of disease incidence and severity. Potassium silicate (@ 4gL^{-1}) also gave the satisfactory result to develop the resistant system in rice plant upon artificial inoculation of pathogen and in foliar spray in field condition. This result is agreement with Datnoff *et al.* (1991), who demonstrated that silicon (Si) containing fertilization on organic rice soils reduced blast incidence 17 to 31% and helmintosporiosis 15 to 32% in relation to a non-fertilized control. Gama *et al.* (2004) studying the effect of Si containing fertilization on soil or leaf against cotton diseases, found reduction of disease incidence in both forms of fertilization. Menzies *et al.* (1992) noticed the formation of a coating on leaves after spraying of potassium silicate, suggesting that the formation of this “film” would strengthen the cuticle activity as a mechanical barrier to pathogen penetration.

Among the chemicals, urine of pregnant cattle treated plots had the highest no. of tiller than others. In pot condition the tiller no was 27.66 cm where in field condition it was 23.40 cm. As the tiller number was higher it also showed higher panicle mass. So, the cattle urine gave beneficial results.

CHAPTER VI

SUMMARY AND CONCLUSION

Rice is the most important crop in respect of Bangladesh. It is the staple food of the people and people of our country are economically very dependent on rice cultivation. The blast disease is one of the major diseases of rice. It works as a major constraint in rice cultivation during boro season. Our environmental condition during boro season and geographical location helps the pathogen to disperse easily. Several studies have been done since the destructive outbreak of blast disease in our country. Chemical fungicides are used to mitigate blast destruction. They are some extent effective. But they are expensive and harmful for the nature. If we can induce resistance in plants from within there will be long lasting effects. And they can be less to non-harmful for the nature. For the study BRRI dhan 28 was selected and different novel chemicals were used at different stages of plant growth.

From the present study, the selected novel chemicals showed different degrees of effectiveness in respect of disease incidence and severity in pot condition. Then they were again tested in field condition to evaluate their effectiveness and it was found almost similar result in pot condition. In pot condition disease incidence (DI) was higher in control ranged 55.77-90% and the lower in T4 (pregnant cattle urine) ranged 0.00-3.5%. In field condition disease incidence range was recorded 3.21-11.20%. The lowest DI was again recorded in T4 (pregnant cattle urine) and the highest in control. In pot condition, the highest disease severity was also recorded in control ranged 33.33-75% and the lowest in T_4 (pregnant cattle urine) ranged 0-4.5%.

In pot condition, the lowest (9.67) number of tiller/pot was recorded in control and the highest (27.66) in T4. In field condition, the highest (23.40) number of tiller/hill was found in T4 (pregnant cattle urine) and the lowest in control (9.73). In pot condition, the highest (25.7) panicle mass/pot was recorded in T4 (pregnant cattle urine) and the lowest (8.67) in control. In field condition, the highest (20.30) panicle/hill was found in T4 (pregnant cattle urine) and the lowest (7.33) in control. In field experiment plant height range was 68.27-82.40 cm and panicle length range was 11.07-24.32 cm. From the current study, it was found that all the selected novel chemicals gave more or less desirable results. But, the pregnant cattle urine (T4) gave the better results in respect of all considering parameters in both pot and field conditions. Hence, it may be concluded that animal product and silicon containing chemicals are effective against blast disease of rice. The main objective was to find a both environment friendly and easily available novel chemical. The cattle urine fits well with the socio-economical condition of our country. It can be beneficial for poor farmers of our country. They will find it easier to adapt to this technology to control the plant diseases. But, further trials are recommended to minimize the errors and establish the doses more accurately.

CHAPTER VII

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