IN-VITRO SUPPRESSION OF WHEAT BLAST PATHOGEN *MAGNAPORTHE ORYZAE TRITICUM BY* ELICITORS

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ABSTRACT

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The present study was conducted under *in-vitro* conditions to investigate the effect of elicitors against *Triticum* pathotype of *Magnaporthe oryzae* causing wheat blast. Isolates of *M. oryzae Triticum* were made from the infected samples collected from south-western wheat growing areas of Bangladesh. The isolates were characterized morphologically based on colony colour, colony size, colony shape and mycelium compactness. Based on morphological features isolate-3 was selected for further experimentation. Three elicitors *viz*. chitosan (0.4%), salicylic acid (9mM) and benzoic acid (9mM) were mixed with culture media before inoculating with the selected isolate of *M. oryzae Triricum* to observe suppressing ability of the elicitors. Antipathogenic ability of the elicitors against *M. oryzae Triticum* was assessed and all the elicitors completely inhibited the mycelial growth of *M. oryzae Triticum*. It indicates the potential of the elicitors for application in the field of wheat blast management.

Key words: Elicitors, *In-vitro*, Isolation, *Magnaporthe oryzae Triticum*, Suppression.

INTRODUCTION

Wheat is one of the world's most important cereal crops and occupying the second position after rice in Bangladesh (Hossain *et al*, 2013). About 2 million farmers have benefited from wheat cultivation and about 600,000 people are employed for a period of 120 man-days during the wheat season in the country (Banglapedia 2014). Wheat is cultivated in about 4.5 lac hectare with a total production of about 13.5 lac m ton (AMIS & BBS 2016). During the early 2016 an outbreak of wheat blast caused by *Magnaporthe oryzae* Pathotype *Triticum* was observed in the southern wheat growing areas of Bangladesh (Malaker *et al*. 2016). The losses due to wheat blast were estimated in the range of 10 to 100% in South American countries (Duveiller *et al*. 2016), where the disease was endemic for 30 years since its first detection in 1985 in Brazil.

The chemical control has low efficiency in controlling blast and in reducing vulnerability to new pathotypes and races. Thus, there is urgent need for developing an effective, economic and eco-friendly method to control *Magnaporthe oryzae Triticum.* Due to increasing concern of environment and health issue, scientists around the globe are now concentrating to exploit plant innate ability to overcome impediments caused by pest and pathogens. Induced resistance can be achieved in plants by different abiotic and biotic stimuli. Elicitors in plant biology are extrinsic or foreign molecules often associated with plant pests, diseases or synergistic organisms. This response results in the enhanced synthesis of metabolites which reduce damage and increase resistance to pest, disease or environmental stress. (Tumpa *et al*. 2017).

Based on the previous findings, it is highly likely that salicylic acid, benzoic acid and chitosan can be used as foliar application that might induce resistance to overcome blast disease as well as enhancing crop production (Mondal *et al.* 2012). Properties of chitosan for inhibition of pathogenic bacteria and fungi in antimicrobial films and edible coatings are used (Yarahmadi *et al*. 2014). These Chitosan can modulate various cellular function including reactive oxygen production, ion channel activity through phosphorylation and dephosphorylation of target protein, stomatal movement, up regulation of pathogenesis related genes (Khokon *et al*. 2010). Salicylic acid (SA) is one of the key hormonal factors determining the fate of plants exposed to stressful conditions, which is naturally found in plants and shown to be involved in the plant defense related actions against infection by various pathogens (Kawano and Bouteau 2013). The effects of benzoic

acid as an allelochemical on seed germination, seedling growth, biochemical parameters, and response of antioxidant enzymes in *Triticum aestivum* L were investigated. This stress exhibited inhibitory effect on growth and metabolism of wheat seedlings (Yadav and Singh 2013). However, use of elicitors as inducer of resistance in wheat against blast pathogen has not yet been reported. The present study was undertaken to investigate the effect of elicitors in reducing mycelial growth of *Magnaporthe oryzae Triticum.*

MATERIALS AND METHODS

Isolation and identification of *Magnaporthe oryzae Triticum*

Wheat blast samples were collected from wheat blast affected area of eight south-western districts of Bangladesh. Infected wheat seeds and other plant parts were placed on wet blotter paper according to ISTA rules for isolation of *M. oryzae Triticum* (ISTA 2010). Mycelia of the fungus were aseptically transferred from seed surface and infected parts to PDA medium. Then, tips of the fungal colony were transferred to other PDA plates and incubated at 25ºC with more than 80% relative humidity for luxuriant growth. Necessary multiplication of the pathogen was done. For the verification of wheat blast pathogen, the wheat plants were artificially inoculated in pot. Mycelial suspensions were prepared with 15 ml distilled water (9 cm culture plates) by collecting the mycelia from the surface of 15 days old PDA culture (Daw *et al.* 2008). The inoculum suspension was sprayed at four growth stages namely seedling stage, stem elongation stage, panicle initiation stage and maturity stage using hand sprayer. The pots were covered with polythene bags for maintaining high moisture for 24 hours (Hossain *et al*. 1992). The appeared symptoms were observed and studied. Then slides were prepared from the infected plant parts to observe pathogenic structure. The pathogen was re-cultured as previous to get pure culture.

Growth study of *Magnaporthe oryzae Triticum*

A 5 mm mycelial disc of the isolates was placed at the center of each plate containing PDA medium. Different morphological characters of the isolates *viz*. Colony color, Radial mycelial growth (cm), Colony shape, colony size and Texture were recorded. Radial growth of the isolates was measured at 24 hours interval for 9 days by the following formula (Ahmed 2003), Mean radial growth = (Length + Breadth)/2. There were three replications for each of the isolates.

Preparation of stock solution

Solid chitosan 0.4 g was dissolved in concentrated acetic acid added drop by drop, then diluted with distilled water up to volume of 100 ml to obtain 0.4% chitosan stock solution. An amount of 12.21 g salicylic acid was dissolved in 100 ml ethanol and stirred by a magnetic stirrer for 5-10 minutes to get 1M stock solution of salicylic acid. To prepare 9mM salicylic acid, 0.9 ml from stock solution was dissolved in 100 ml distilled water. Solid benzoic acid 13.81 g was dissolved in 100 ml ethanol and stirred by a magnetic stirrer for 5-10 minutes to get 1M stock solution of benzoic acid. To prepare 9 mM benzoic acid, 0.9 ml from stock solution was dissolved in 100 ml distilled water.

In-vitro antagonism assay

To determine the growth suppressing ability against the test fungus, chitosan (0.4%), salisylic acid (9 mM) and benzoic acid (9 mM) were added to PDA medium at 1 ml solution for each PDA plate and stirred for 10 minutes to get homogenous mixture. Then a 5 mm diameter fungal disc of isolate-3 was placed in the center of each petri-dish. Petri-dishes were incubated at 25^0C. Control plates were maintained. Fungal colony diameter in all plates was recorded at 24 hours interval for nine days. There were three replications for each treatment.

Data analysis

Mycelial growth was measured by a measuring scale (mm) for nine days at 24 hours interval. The collected data were analyzed statistically by using Web Agri Stat Package (WASP) program. The significance of the difference among the means was compared by LSD (Least Significant Difference) test.

RESULTS AND DISCUSSION

Isolation and purification of *Magnaporthe oryzae Triticum*

To verify the organism as *Magnaporthe oryzae Triticum*, isolation and purification was done from artificially infected wheat plants. In order to do so, PDA medium fortified with akashmoni leaf extract was used as growing medium. Gray to white mycelia was observed after placing the infected plant parts on medium. Profuse growth was observed in akashmoni medium compared to PDA medium (Figure 1).

Figure 1. Pure culture of *M. oryzae Triticum* in PDA medium (A) and akashmoni leaf extract with PDA medium (B)

Symptoms of wheat blast disease

Artificial inoculation of wheat seedling leaves using mycelia of the isolates produced characteristic

symptoms seven days after inoculation. Initially, a diamond shaped, water soaked lesions on green leaves were observed, which gradually turned into an eye shaped lesion, with a tan or gray colored center (Figure 2 A). At a later stage, the spots enlarged, spread to entire leaves, and killed the leaves. Infected heads became bleached and produced shriveled seeds or no seed at all (Figure 2 B). Spindle shaped 2-septate conidia were found from the infected plant parts under Microscope (Figure 2 C). Infected plant parts were placed on PDA medium by following Tissue planting method (Figure 2 D and E). The pure culture prepared from the infected plant parts was same as previous pure culture (Figure 2 F).

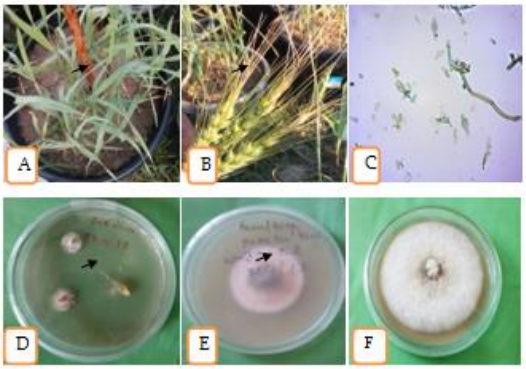

Figure 2.A. Infected leaves, B. Infected heads, C. Conidia observed under microscope, D. Infected parts inoculated in PDA medium, E. Sub culture, & F. Pure culture of *Magnaporthe oryzae Triticum*

Morphology of different isolates of *Magnaporthe oryzae Triticum*in PDA medium supplemented with akashmoni leaf extract

Morphological characters of three isolates of *Magnaporthe oryzae Triticum* grown in PDA medium supplemented with akashmoni leaf extract are presented in Table 1. Morphological characteristics including colony colour (upper side and down side), colony size, colony shape, and compactness were recorded for each of the isolates. Isolate-1 showed regular size, circular shape and slightly compact mycelia with black center surrounded by whitish upper side and light black down side. Isolate-2 showed regular size, round shape and compact mycelia with ashy center surrounded by whitish upper side and light black down side. Isolate-3 showed regular size, circular shape and slightly compact mycelia with gray center surrounded by white upper side and light black down side. Pictorial view of the three isolates is shown in Figure 3.

Table 1. Morphological characters of three isolates of *Magnaporthe oryzae Triticum* in PDA medium supplemented with akashmoni leaf extract

Name of the isolates	Colony colour		Colony size	Colony shape	Compactness
	Upper side	Down side			
Isolate -1	Center black, surrounding whitish	Light black	Regular	Circular	Slightly compact
Isolate -2	Center ash, surrounding whitish	Light black	Regular	Round	Compact
Isolate -3	Center gray, surrounding white	Light black	Regular	Circular	Slightly compact

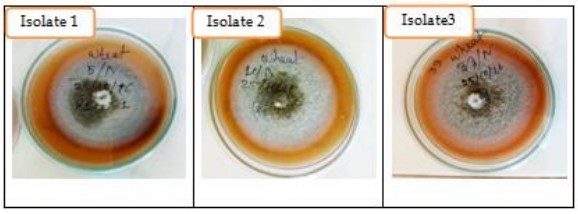

Figure 3. Different isolates of *Magnaporthe oryzae Triticum* in akashmoni supplemented PDA culture

Variation in mycelial growth of the isolates of *Magnaporthe oryzae Triticum*

The mycelial growth of the different isolates of *Magnaporthe oryzae Triticum*is is presented in Table 2. The mycelial growth of three isolates differed significantly and ranged from 9.40 to 10.50 mm at 24 hours, 18.10 to 19.50 mm at 48 hours, 27.30 to 28.50 mm at 72 hours, 34.40 to 37.60 mm at 96 hours, 43.30 to 47.20 mm at 120 hours, 48.30 to 52.40 mm at 144 hours, 56.80 to 58.60 mm at 168 hours, 65.80 to 64.40 mm at 192 hours and 74.20 to 76.50 mm at 216 hours. The mycelial growth of all the isolates increased with the time after inoculation of the growth medium. The maximum mycelial growth was recorded in the isolate-3 followed by isolate-2 and the minimum mycelial growth was observed in the isolate-1.

Table 2. Mycelial growth of the isolates of *Magnaporthe oryzae Triticum*

Isolates	Radial mycelial growth HAI (mm)								
	24	48	72	96	120	144	168	192	216
Isolate 1	9.40 b	18.10 c	27.30 b	34.40 c	43.30 c	48.30 c	56.80 c	65.80 c	74.20 c
Isolate 2	9.30 b	18.80 b	27.50 b	36.30 b	45.30 b	49.60 b	57.70 b	66.60 b	75.50 b
Isolate 3	10.50 a	19.50 a	28.50a	37.60 a	47.20 a	52.40 a	58.60 a	68.40 a	76.50 a
$LSD_{(0.05)}$	0.34	0.26	0.34	0.34	0.34	0.34	0.28	0.28	0.28
CV (%)	1.77	0.68	0.63	0.48	0.39	0.35	0.24	0.21	0.19

Note: HAI= Hour After Inoculation

In-vitro* growth suppressing ability of the elicitors against *Magnaporthe oryzae Triticum

Growth suppressing ability of elicitors on radial mycelial growth of *Magnaporthe oryzae Triticum* over control was assessed at 24, 48, 72, 96, 120, 144, 168, 192 and 216 hour of inoculation of the growth medium (Table 3). In case of control (respective solvent), the mycelial growth of the pathogen increased from 8.50 mm at 24 h to 70.00 mm at 216 hour of inoculation, while no growth was found with any of the elicitors even after 216 hour of inoculation. All the elicitors completely inhibited the mycelial growth of *Magnaporthe oryzae Triticum*in PDA medium (Figure 4).

Table 3. *In-vitro* growth suppressing ability of the elicitors against *Magnaporthe oryzae Triticum*

Treatments	Radial mycelial growth HAI (mm)								
	24	48	72	96	120	144	168	192	216
T_1	8.50	13.50	25.00	35.00	46.00	52.00	57.00	64.00	70.00
T_2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T_3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
T_4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

Note: T_1 = Control, T_2 = Chitosan (0.4%), T_3 = Salicylic acid (9mM), T_4 = Benzoic acid (9mM), HAI= Hour After Inoculation

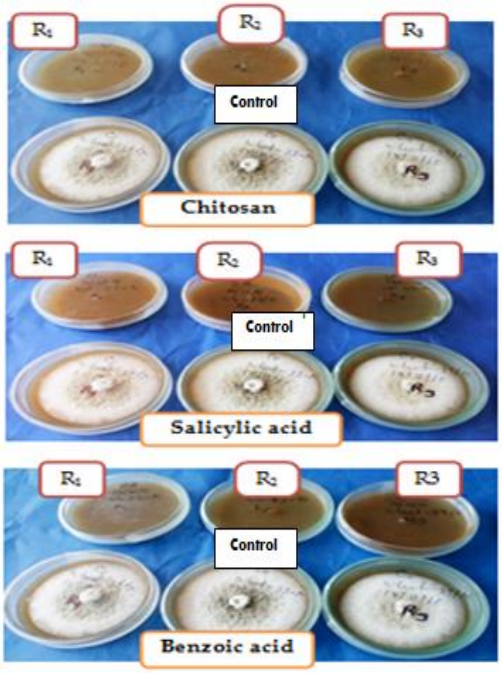

Figure 4. *In-vitro* growth inhibition of *Magnaporthe oryzae Triticum* by elicitors

In the present study, morphological characters of different isolates of *Magnaporthe oryzae Triticum* and the effects of different elicitors *viz*. chitosan, salicylic acid and benzoic acidas antifungal activity against *Magnaporthe oryzae Triticum* were studied. The morphological characteristics including colony colour, colony size, colony shape, and compactness were recorded. Three isolates collected from different regions showed difference in colony morphology and mycelial growth. Isolate-1 showed regular size, circular shape and slightly compact mycelia with black center. Isolate-2 showed regular size, round shape and compact mycelia with ashy center. Isolate-3 showed regular size, circular shape and slightly compact mycelia with gray center. Mycelial growth varied significantly among the three isolates with the minimum recorded in isolate-1 and the maximum in isolate-3. The findings are consistent with those obtained in the studies of Gashaw *et al*. (2014), where they reported morphological variability in different isolates of *Pyricularia grisea (Magnaporthe grisea)* characterized based on cultural, morphological and physiological characters.

The elicitors completely inhibited the mycelial growth of *M. oryzae Triticum* in PDA medium. A sharp

increase of mycelial growth with the increase in incubation period was observed in the control (without elicitors), whereas no growth of the pathogen was found when chitosan (0.4%), salicylic acid (9mM) or benzoic acid (9mM) was added to the growth medium. Similarly, Jabnoun-Khiareddine *et al.* (2015) reported the efficacy of chitosan and salicylic acid for antifungal activity against ten tomato phytopathogenic fungi i.e. *Fusarium oxysporum* f. sp. *lycopersici, F. oxysporum* f. sp. *radicis lycopersici, F. solani, Verticillium dahliae, Rhizoctonia solani, Colletotrichum coccodes, Pythium aphanidermatum, Sclerotinia sclerotiorum, Botrytis cinerea,* and *Alternaria solani.* Chitosan (0.5-4 mg/ml) and salicylic acid (1-25 mM) inhibited mycelial growth of all pathogens in PDA medium in a concentration dependent manner, with the greatest inhibition achieved using the highest chitosan and salicylic acid concentrations. Tumpa *et al*. (2018) reported superior performance of chitosan (2000ppm) and yeast elicitors (2000ppm) in suppressing the growth of seed-borne fungi of cucurbitaceous vegetables in blotter paper method and Tumpa *et al*. (2017) reported complete inhibition of mycelial growth of different fungi with chitosan and yeast elicitors both used at 1000ppm and 2000ppm. Yoon *et al.* (2012) found benzoic acid inhibited mycelial growth of *M. oryzae, Rhizoctonia solani, Sclerotinia sclerotiorum,* and *C. capsici* with minimum inhibition concentration (MIC) values ranging from 62.5 to 125 µg/ml.

The results of the present study indicate that chitosan (0.4%), salicylic acid (9mM) and benzoic acid (9mM) are effective in suppressing the growth of *Magnaporthe oryzae Triticum*. Chitosan, salicylic acid and benzoic acid are bio-polymers and not harmful for ecosystem and are completely safe for human health. Therefore, these bio-polymers can be utilized as alternative to chemical fungicides for wheat blast management. However, intense researches are needed to develop suitable formulations before suggesting these bio-chemicals for field application.

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