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Soil supplementation with Si, B and Zn and their synergetic effects in reducing severity of wheat blast (*Magnaporthe oryzae* Triticum)

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ABSTRACT

Wheat blast (Magnaporthe oryzae Triticum) in Bangladesh and South America is recognized as one major limiting factor of wheat production. Its control using chemical pesticides raises concerns about food safety and pesticide resistance, which have dictated the need for alternative blast management approach, nutrient supplementation could be an ecofriendly alternative. Experiments were carried out under confined net house condition for two consecutive cropping seasons. Single doses of the nutrients (Si, B and Zn) were incorporated during soil preparation. Plants of the wheat blast susceptible variety BARI Gom-26 were inoculated with spores (1 x 107 spores ml-1) of Magnaporthe oryzae Triticum at blast vulnerable pre-heading stage of 52 days age. Typical wheat blast symptoms of spike bleaching from top to downward appeared on sight 14 days after inoculation i.e., 66 days age of the crop. Incidence and severity of blast bleaching of spike were scored for four times starting from 68 days age @ three day's interval. None of the nutrients could stop the incidence of blast on wheat; however, some nutrients reduced the blast incidence significantly. Solo application of Si, B and Zn or combination of two caused significant reduction of spike bleaching. With the mixed application of Si, B and Zn, > 47% reduction of wheat blast severity was obtained. The results revealed that the soil application of silicon. zinc and boron had a synergistic effect on the intensity of blast disease of wheat.

Keywords: Wheat blast, Nutrient Supplementation, Management

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Introduction

Wheat blast was first observed in Bangladesh in the southern districts in the middle of February of 2016 (Aman, 2016; BARI, 2016). It affected about ~20% of Bangladesh's total wheat producing area in 2015-16, presenting a significant threat to the country's aggregate wheat production (Meah *et al.*, 2016).

The warm and humid weather of Bangladesh is very much favorable for the epidemic development of wheat blast disease. The pathogen is seed borne, air borne and may be disseminated by rain splash (Ceresini *et al.*, 2018). A great amount of crop loss occurs if fungal infection occurs at the base of the rachis, which inhibits the development of grains and causes destruction of spike (Kohli *et al.*, 2011).

Wheat varieties available in Bangladesh are all highly susceptible to blast; no foliar fungicide spray is effective once the infection is already established in the plant head. However, BWMRI (Bangladesh Wheat and Maize Research Institute) released wheat variety BARI Gom 33 in 2019-20 cropping season which is enriched with Zn and considered to be resistant to blast disease (Hossain *et al.*, 2019).

Plant uptakes nutrients from soil and maintains its growth and development. Deficiencies of the nutrients affect tissue hardiness making them delicate and thus plants become weak and thereby susceptible to invading microbes. Thus, nutrients can affect disease resistance or tolerance. A healthy plant is able to limit the penetration, development and reproduction of the invading pathogens (Graham and Webb, 1991). Therefore, soil amendment with proper nutrients is important for helping plant maintain tissue hardiness and in that way to control plant disease in an integrated disease management approach (Huber and Graham, 1999; Graham and Webb, 1991). Therefore, new strategies for disease control need to be urgently investigated. Silicon application has been found a viable method of enhancing wheat resistance against blast (Rafi and Epstein, 1999; Filha *et al.*, 2011; Debona *et al.*, 2012) and root and foliar diseases of several other crops (Datnoff *et al.*, 2007).

Boron strengthens the structural integrity of the cell walls and cell membranes (Marschner, 1995). Plants also utilize boron as a physical "binding agent" as an alternative bridge for cell wall integrity and as a regulator of the pentose phosphate pathway, which is responsible for the production of resistance compounds (Marschner, 1995; Brown *et al.*, 2008).

It is believed grey leaf spot of coconut and guava anthracnose diseases are related to zinc deficiency. Soil application or foliar spray of zinc proved effective in reducing the diseases. The integrity of cellular membranes also requires Zn to preserve the structural orientation of macromolecules and keep ion transport systems (Cakmak, 2000; Kabata-Pendias and Pendias, 2001; Alloway, 2004; Disante *et al.*, 2010).

Therefore, Si, Zn, and B when present in plant cells in optimum conditions contribute to maintaining cell thickness and cell integrity and thereby help the plants in resisting invading pathogens. It is assumed supplementation of such elements might complement the deficiency and enhance the defense mechanisms of the wheat plants against *Magnaporthe oryzae* Triticum, the blast pathogen.

Evidence is there in favor of this proposition as obtained from research done in some countries even with significant results achieved for wheat blast. However, no information is available in Bangladesh on the use of nutrients like Si, B and Zn as soil amendments for the management of blast incidence in wheat. This research is, therefore, a first- time attempt to investigate the effect of soil amendment with certain nutrients such as Si, B and Zn on the reduction of severity of blast incidence in wheat.

Materials and Methods

Net house experiments were carried out in the BINA (Bangladesh Institute of Nuclear Agriculture) farm. Laboratory works were accomplished in the IPM Lab of Bangladesh Agricultural University (BAU). The experiments were carried out during the two consecutive wheat cropping seasons (2018-19, 2019-20). Wheat variety BARI Gom-26, susceptible to wheat blast was used for the experiment.

Nutrient elements and minerals

Three plant nutrients such as Silicon, Boron and Zinc were applied at single doses (recommended) (BARC, 2018) in the 1st year i.e., cropping season of 2018-19 experiment. In the second year (2019-20), the three nutrients/minerals were applied in combination of two to three @ single higher dose of each (Table 1).

Nutrient	Commercial Name with active ingredient (%)	Quantity mixed with soil (g per 16L pot)	Doses applied* (Kg ha ⁻¹)
Si (Silicon)	Super Silica Silica- 71%	0.7280 and 1.4550	50.0 and 100.00
Zn (Zinc)	Zinc Sulphate Mono Zn-36%	0.1090 and 0.1673	7.50 and 11.25
B (Boron)	Bingo (Borax) Solubor B-20%	0.0437 and 0.0873	3.00 and 6.00

Table 1. Nutrients and minerals incorporated in the soil.

*In the 1^{st} year (cropping season 2018-19), lower doses of the nutrients were applied while the higher dose was applied in the 2^{nd} year (2019-20).

In the 1st year experiment, there were four treatments including control. In the 2nd year experiment, there were five treatments including control. The treatments were arranged following Completely Randomized Design (CRD) with three replications. Plastic pots of 16L size were poured with soil mixed up with cow dung and recommended fertilizers (BARC, 2018) and prepared for seed sowing.

Mixing nutrients with pot soil

As per recommendation (BARC, 2018), double quantity of each of the nutrients were mixed during pot soil preparation. The quantities were 45.5 and 90.9; 6.8125 and 10.4560; 2.731 and 5.456 mg kg soil⁻¹ for Super Silica, Zinc Sulphate and Borax, respectively for lower and higher

doses (Table 1). The nutrients were mixed either single (Table 2 & 3) or a mixture of two or three (Table 4 & 5) as per design of the experiment.

Sowing of seed

Thirty apparently healthy seeds of wheat variety BARI Gom26 were sown in each pot.

Inoculation of the wheat plants with MoT at preheading stage

Pure culture of *Magnaporthe oryzae* Triticum (MoT) was collected from IPM Lab, Bangladesh Agricultural University. The culture was multiplied in Oatmeal agar media. The plates were incubated at $30 \pm 1^{\circ}$ C with continuous NUV light (650 lux) for 15-20 days for sporulation (Fig. 1).



Fig. 1. (A). Culture (20 days old) and (B). typical 2-septate 3-celled pyriform spores of *Magnaporthe oryzae* Triticum (microscopic view 40x).

Density of the spores was calculated by harvesting the conidia/mycelia by flooding the Petri dish with 5 ml of sterile distilled water and dislodging the conidia with a bent glass rod. Spore density was 1×10^7 per ml as calculated in Hemocytometer count (Akagi *et al.*, 2015). The germination ability of the spores was checked through continued microscopic observation of the slide prepared out of spore suspension.

At pre-heading stage, (52-53 days after sowing), the plants of wheat variety BARI Gom-26 were inoculated with spores of MoT. There were 15 plants maintained per pot and thus 45 plants per treatment. All parts of wheat plants i.e., leaves and emerging spikes were inoculated with MoT suspension. The inoculated plants were kept under polythene cover in >80% moisture condition and temperature of $30 \pm 1^{\circ}$ C. Spore suspension of MoT was sprayed onto wheat plant parts with the help of an atomizer (Small hand sprayer).

After inoculation, the plants in the pots were covered with polythene shed to maintain 100% relative humidity and to keep it in darkness (Fig. 2). A polythene shed was erected on an area of 6 m x 15 m with support from all four sides and overhead, the height was maintained as 2.5 m. The polythene was white, transparent and 2 mm thick.



Fig. 2. Polythene shed covering experimental plot in Bangladesh Institute of Nuclear Agriculture (BINA) campus farm.

Sprinkler irrigation facilities were installed inside the shed from two sides for watering the plants to maintain the moisture. As Barksdale and Asai (1961) illustrated, conidiation requires a period of darkness and conidia are released in a diurnal pattern. Temperature and moisture were monitored by an automatic reader (Thermo-Hygrometer Min-Max SH-110). The polythene cover was kept over and taken down during day and nighttime, respectively. It was continued for 14 days to see the first expression of blast symptoms when the cover was removed finally.

Data collection

The plants were kept under continued observation for expression of wheat blast symptoms. Both leaves and spikes were observed regularly for blast infection. Data on the following parameters were recorded: i). Days to symptom expression, ii). Disease incidence, iii). Disease severity and iv). Yield. The incidence of wheat blast and its severity were scored for four times @ three days interval starting at 68 days age of the plants i.e., 2 days after symptoms expression.

Disease Incidence: Number of spikes infected per replication expressed in percentage (Rajput and Bartaria, 1995). Blast incidence (%) = $Pi/Pt \times 100$ where, Pi = Number of spikes infected, and Pt = Total number of spikes counted.

Disease Severity: Percent surface area of spike infected/bleached was estimated. Ten spikes were selected randomly per pot and tagged. Percent spike surface area bleached was determined through eye estimation and average of 10 readings was calculated for one pot (replication).

Statistical analysis

The collected data were analyzed statistically by using Minitab 17 software. Nutrient treatments were considered as factor, Disease Incidence (%DI), Disease Severity (%DS) and yield (g/treatment) as variables. Treatments mean squares and error mean squares were calculated. A one-way ANOVA (Analysis of variance) table was constructed for F-test. With the significant Fvalue (P =0.05), the treatment means were compared by Fisher Pairwise Comparison at LSD (P=0.05) (www.minitab.com: Minitab Statistical Software).

Results and Discussion

Effect of soil supplementation of different nutrients on incidence of blast of wheat

Incidence of blast

Typical symptoms of wheat blast expressed as bleaching from top of the spike was first noticed in the untreated plants 14 days after inoculation with wheat blast pathogen *Magnaporthe oryzae* Triticum (Fig. 3).



Fig. 3. Spike bleaching top to downward (20 days after inoculation, 73 days age) in untreated wheat plants of variety BARI Gom 26 inoculated with *Magnaporthe oryzae* Triticum during the cropping season of 2019-20.

Untreated plants experienced significantly higher percent spike bleaching than all treatments. Among the treatments, Si treated plants had significantly lower spike infection. Silicon in soil reduced spike infection by 48.5% while wheat plants in B amended soil had 36.5% less blast incidence. Zn treated plants had significantly higher blast incidence than Si and B treatments (Table 2).

Table 2. Incidence of blast in wheat plants grown in soil amended with Si, Zn and B during the cropping season of 2018-19 under confined condition in the Bangladesh Institute of Nuclear Agriculture campus farm.

Treatment	Disease Incidence (%)						
Name	68 DAS	71 DAS	74 DAS	77 DAS			
Control	23.46 a	53.20 a	78.84 a	98.33 a			
Silicon	12.14 b	22.07 C	30.40 c	50.67 d			
Zinc	21.81 a	50.49 a	66.86 a	76.14 b			
Boron	17.44 a	35.78 b	52.58 b	62.49 c			
CV (%)	22.600	12.860	11.054	4.829			
LSD (0.05)	8.925	10.340	11.980	6.910			

Control: no nutrients, Si: 45.5 mg kg soil⁻¹, B: 2.731 mg kg soil⁻¹, Zinc: 6.8125 mg Kg⁻¹. DAS: Days After Sowing.

Severity of blast

Soil amendment with nutrients had significant influence on the area of spike bleached i.e. blast severity. At the beginning of infection, B and Zn treated plants had spike bleaching similar to that of control while Si treatment caused significantly lower percent spike bleaching. This trend continued until final day of observation. On the final day recording, three nutrients produced significantly lower blast severity than that of control. Silicon amended soil reduced blast severity by 38.6% (Table 3, Fig. 4).

Table 3. Severity of wheat blast as affected by soil incorporation of Si, Zn and B during the cropping season of 2018-19 under confined condition in the Bangladesh Institute of Nuclear Agriculture campus farm.

Treatment	Disease Severity (%)					
name	68 DAS	71 DAS	74 DAS	77 DAS		
Control	32.00 a	57.67 a	81.00 a	99.33 a		
Silicon	12.87 b	23.33 b	42.00 c	61.00 b		
Zinc	27.20 a	44.67 a	63.00 b	77.33 b		
Boron	26.67 a	44.30 a	56.33 b	72.00 b		
CV (%)	24.69	18.02	11.90	10.59		
LSD (0.05)	13.73	18.78	16.71	18.44		

Control: no nutrients, Si: 45.5 mg Kg soil⁻¹, Zinc: 6.8125 mg Kg soil⁻¹, B: 2.731 mg Kg soil⁻¹. DAS: Days After Sowing.

Effect of soil incorporation of nutrient mix on the incidence and severity of blast disease of wheat

The nutrients were incorporated in the soil in mixture of two or three during pot soil preparation. Treatment effects on the disease incidence of blast of wheat were recorded at 3-day interval starting from 68 DAS (days after sowing) i.e. two days after 1st sign of disease expression and then it was continued to 77 DAS.

Incidence of Blast

At 68 to 77 DAS, the lowest disease incidence was recorded in case of T4 treatment. Moderate effects were observed in T_1 , T_2 , and T_3 treatments and the highest disease incidence was recorded in case of control. T_4 treatment i.e., combined application of Si, B and Zn produced significantly better effect than all other treatments where blast incidence was reduced by 49.22% over control at 77 DAS (Table 4, Fig. 4).

Table 4. Incidence of wheat blast in response to nutrient mix incorporated in soil during the cropping season of 2019-20 under confined condition in the Bangladesh Institute of Nuclear Agriculture campus farm.

Treatments	Disease Incidence (% spike bleached)							
	68 DAS	71 DAS	74 DAS	77 DAS				
To	33.59 a	48.72a	66.40a	100.00a				
T_1	28.32 a	37.92 a	49.81 b	71.16 b				
T_2	29.63 a	37.26 a	49.78 b	62.40 b				
T ₃	19.10 b	33.72 b	40.55 b	58.21 b				
T ₄	12.59 b	22.51 b	37.04 b	50.78 c				
CV (%)	23.63	17.84	14.22	13.52				
LSD (0.05)	12.38	12.65	13.87	19.19				

 T_0 : no nutrients, T_1 : Silicon 90.9 mg Kg soil⁻¹, T_2 : Silicon 90.9 mg Kg⁻¹ + Zinc 10.456 mg Kg soil⁻¹, T_3 : Silicon 90.9 mg Kg⁻¹ + Boron 5.456 mg Kg soil⁻¹, T_4 : Silicon 90.9 mg Kg⁻¹ + Zinc 10.456 mg Kg⁻¹ + Boron 5.456 mg Kg soil⁻¹. DAS: Days After Sowing.



Fig. 4. Wheat plants displaying different levels of spike bleaching inoculated with *Magnaporthe oryzae* Triticum and grown in Si, B and Zn amended soil at 67 days age (14 days after inoculation) during the cropping season of 2019-20. A). Control: untreated and inoculated, B). SiD₂: Soil treated with Silicon (Dose 2), C). BD₂: Soil treated with Boron (Dose 2), D). ZnD₂: Soil treated with Zinc (Dose 2), E). Si + Zn +B: Soil treated with mixture of Si, Zn and B (2019-20).

Increase in Blast disease severity of wheat with time under soil condition amended with nutrient mix.

In the figure 5, it is seen severity of blast increased with time in all the five cases. Solo application of Si (T_1) slowed down the rate of increase in blast severity compared to that of control. Addition of Zinc had similar effect that of Si. However, the rate of increase of blast severity was significantly reduced for addition of Boron. The three nutrients when incorporated in the soil together (T_4), the effect was significantly different; the blast severity was significantly



Fig. 5. Effect of soil incorporation of nutrient alone or in mix on the progress of the severity of blast on wheat variety BARI Gom 26 under confined inoculated condition during the cropping season of 2019-20. T₀: no nutrients, T₁: Silicon, T₂: Silicon + Zinc, T₃: Silicon + Boron, T₄: Silicon + Zinc + Boron. DAS: Davs After Sowing.

Uninoculated wheat plants which did not develop blast produced significantly higher yield than all treatments (Table 5). An increase of yield by95.65% was achieved over control (inoculated) through reduction of blast severity by 47.33%. This was possible through mixed soil incorporation of Si, Zn and B. If reduction in lower than that of three other treatments and untreated plants (Fig. 5).

Nutrient supplementation and yield of wheat under blast condition

Blast severity affected yield of wheat significantly. Yield was drastically reduced with increase in blast severity. With supplementation of nutrients, reduction in blast severity was occurred leading to corresponding increase in yield of wheat (Fig. 6).



Fig. 6. Effect of reduction on wheat blast severity (%DS) on the increase of yield over inoculated control. DS: Disease Severity (% spike area bleached).

percent blast severity could be achieved up to 50%, approx. 100% increases in yield would have been obtained (Fig. 7). Considered the yield of uninoculated healthy plants, yield of inoculated untreated plants was reduced by 95.02% and with supplementation of nutrients,958.18% of the yield loss could be recovered (Table 5, Fig. 8).

Table 5.	Yield of wh	heat as	affected	by blast	t severity	and	its	recovery	though	soil	amendment	with
	nutrients i	n the cro	opping se	eason of	2019-20.			•	C C			

	Treatment	Disease Severity (DS) (%)	Reduction in DS over inoculated control (%)	Yield* (g per treatment)	Yield Reduction over control (uninoculated healthy) (%)	Yield Recovery over control (inoculated untreated) (%)	Yield increase over control (inoculated untreated) (%)
To	Control (inoculated untreated)	100.000a	00.00	1.653e	95.02	-	-
T ₀₁	Control (uninoculated healthy)	0.000d	00.00	33.210a	-	-	190.84
T ₁	Si	70.667b	29.33	11.900d	64.17	621.21	61.97
T_2	Si + Zn	70.000b	30.00	12.550cd	62.21	660.60	65.94
T ₃	Si + B	61.000bc	39.00	13.983c	57.90	747.27	74.58
T_4	Si + Zn + B	52.670c	47.33	17.463b	47.43	958.18	95.65
	CV (%)	8.724		5.071			
	LSD (0.05)	13.4650		1.7792			

 T_0 : no nutrients (inoculated), T_{01} : nutrients (not inoculated), T_1 : Silicon 90.9 mg Kg soil⁻¹, T_2 : Silicon 90.9 mg Kg⁻¹ + Zinc 10.456 mg Kg soil⁻¹, T_3 : Silicon 90.9 mg Kg⁻¹ + Boron 5.456 mg Kg soil⁻¹, T_4 : Silicon 90.9 mg Kg⁻¹ + Zinc 10.456 mg Kg⁻¹ + Boron 5.456 mg Kg soil⁻¹, T_4 : Silicon 90.9 mg Kg⁻¹ + Zinc 10.456 mg Kg⁻¹ + Zinc 10.456 mg Kg⁻¹ + Zinc 10.456 mg Kg soil⁻¹, DS: Disease Severity (% spike area infected). *Average of 3 x 16L pot (replication).



Fig. 7. Relationship between percent reduction in wheat blast severity and percent yield increase under soil supplementation of Si, Zn and B (cropping season 2019-20).

Wheat blast (*Magnaporthe oryzae* pv Triticum) caused heavy loss in wheat production in the south-western region of Bangladesh in 2016. It continued to reappear in the same region regularly every year until 2020 (Anonymous, 2020). Since its first appearance in Brazil in 1985, efforts for developing disease management technique have been continued. Developing resistant capacity within wheat plant against blast is a modern thought; nutrient application is one of the important parts of it.

The research findings of the present study have support of the findings of Cruz *et al.* (2015) confirming positive contribution of Si in augmenting the resistance to blast when a reduction of 42% severity of wheat blast was obtained. The findings also concede the opinion of Silva et al. (2015) reporting reduced fungal growth under increased Si concentration. According to them, Si is involved in the potentiation of the biosynthetic pathway of flavonoids that increases wheat resistance to blast. In addition, silicon acts as a mechanical barrier against fungal appressorial penetration, which is known as physical resistance (Hayasaka et al., 2008). Conversely, under Si deficient soil condition blast severity is severe (Kim et al., 2002; Rodrigues et al., 2003; Ranganathan et al., 2006). Our results comply with all these reports when a reduction of 42% in the incidence of wheat blast was obtained under Si enriched soil condition.



Fig. 8. Recovery of yield of wheat under reduced blast severity (%DS) through supplementation of Si, Zn and B in mixture in pot soil condition during 2019-20 cropping season.

Our results with B are in line with the findings of Broadley *et al.* (2012) stating that boron has a beneficial effect on reducing disease severity through promoting rigidity of the cell wall and, therefore, supports the shape and strength of the plant cell. We observed that boron application in soil reduced the blast incidence in wheat; higher dose produced reduced blast severity. B deficiencies can impair crop quality and reduce yields (Davarpanah *et al.*, 2016); our results are in line with this report as the yield of wheat in B enriched soil increased significantly.

In our experimental field, we recorded lower blast disease incidence in Zn treated plants. The research findings have support from the findings of Wadhwa *et al.* (2014) who found that soil application of Zn as soil-nutritive agent, played an important role in defense mechanism and provided resistance against plant disease. We observed that there was a significant difference between two doses of Zn, higher dose of zinc showed lower disease infection. This finding has support of Khaing *et al.* (2014) reporting that balanced Zn application increased the phenol contents of plant and reduced the severity of rice sheath blight and crown root rot disease in wheat.

Solo application of Si slowed down the rate of increase in blast severity from the start of the blast infection. Addition of Zinc or Boron further reduced the level of severity, but the difference between the effects of solo application of Si and addition of B or Zn on blast severity were not statistically significant. However, the striking thing is three nutrients when incorporated in the soil together the effect was significantly different, the blast severity was significantly lower than that of untreated plants. This might be explained as the synergistic effect of Si, B and Zn. This proposition is supported by the findings of Mahmoud et al. (2020) who reported the plant increase in growth, physiological parameters and endogenous elements (N, P, K, Ca) promoting the crop productivity and thereby host defense under combined application of Si, B and Zn.

In the untreated control plants, blast infection was severe (nearly 100%). The spikes were completely bleached leaving some shriveled and distorted grains. Consequently, the yield was drastically reduced. Si alone increased vield by 62%, addition of B or Zn further increased in yield by 4 to 13% while the synergistic effect of the three nutrients Si, Zn and B caused yield increase by 96%. The results indicate the role of the nutrients specially Si in strengthening defense mechanism of wheat plants when blast severity was reduced, and yield was increased. The findings are in line with Pagani et al. (2014) who reported 92% increase in yield of wheat through soil incorporation of Si in a blast affected field trial.

Conclusion

The findings indicate that addition of nutrients Si or Zn or B has decreased the incidence and severity of blast disease in wheat. Si produced significantly better effect. Soil mixing of Si, Zn and B together produced synergistic effect when blast incidence was reduced by 47% and yield was increased by 96%. It may be concluded that combined application of Si, Zn and B can reduce disease to an acceptable level, or at least to a level at which further control by other cultural practices are more successful, less expensive and environment friendly.

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