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Evaluation of Fungicides for the Control of Bakanae Disease of Rice Caused by *Fusariummoniliforme* (Sheldon)

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ABSTRACT

Bakanae is one of the major diseases of rice in Bangladesh and causes substantial yield loss. To control the disease eleven fungicides from different fungicidal groups were evaluated for their efficacy against seed-borne fungus, *F. moniliforme* as seed treatment both in the laboratory and in the seed-bed during 2011 and 2012. In the laboratory the fungicides were evaluated at three concentration levels viz 2.5, 3.0 and 3.5 gm/L/kg seeds. Overall performance of the fungicides was found to be better in suppressing the seed-borne *F. monilifome* and increasing seed germination. Among the fungicides, Bavistin, Sunphanate, Nativo and Carzeb completely inhibiting the growth of *F. moniliformeinvitro* condition at their low (2.5 gm/L) concentration. Efficacy of the fungicides reduced to some extent when the treated seeds were washed with fresh water except Bavistin. Maximum (85%) average germination was found in Bavistin and Greenzeb treated seeds. The promising fungicides further evaluated to control seedling infection at low (2.5 gm/L) concentration and resulted significant reduction of bakanae infected seedlings in pot soil and in seed bed.

Key words: Fungicides, bakanae disease, rice, control

INTRODUCTION

Bakanae disease of rice caused by *Fusariummoniliforme* (Sheldon) [Teleomorph-*Gibberellafujikuroi*] is one of the major rice diseases in many rice growing countries including Bangladesh (Miah*et al*., 1985; Singh and Sunder, 1997). In Bangladesh the disease was first detected in 1953 by the Mycology and Plant Pathology Division of Bangladesh Agricultural Research Institute and was mostly localized in Aus season (Anon, 1958). Now-a-days it is spread all the seasons and the incidence and severity are quite serious (Momotaz, 2005). The most striking symptom of this disease is yellowing and abnormal elongation of infected rice seedling, which led to the name bakanae. The other symptoms of this disease are foot rot, seedling rot, grain sterility and grain discoloration (Ou, 1985). Even in open field nurseries, one of the problems encountered by farmers in the control of bakanae disease is the difficulty in distinguishing infected seedlings from healthy plants, because there are no obvious symptoms of infected seedlings except the height or slight pale yellowing at the stage of transplanting (Kim, 1981).Under favourable conditions, it causes substantial damage and as high as 95.40% yield loss has been estimated in India (Sigh and Sunder, 1997), 15% in Thailand (Ou, 1985), 20-50% in Japan (Ito and Kumara, 1931) and 21-26.7% in Bangladesh (Latif*et al.,* 2006). The pathogen can be both seed-borne and soil borne. But the role of infected seed as a source of inoculum for this disease is well documented (Santos, 1957; Nath*et al.,* 1970; Winter *et al.,* 1974) and is also the main means of spreading the disease from field to field. Generally, the seed-borne inocula provide initial foci for seedling infection. Infected plants in the field, that reach heading produce enormous amount of inocula, which infect the developing grains results in deep infection on the seed(Kim, 1981).

Bakanae disease of rice recently gained economic importance due to large scale cultivation of susceptible rice varieties. Therefore, it is very much important to manage the disease. The other way to secure the crop from this disease is fungicidal seed treatment. It is found that untreated seeds were heavily infected with an overall 94.5% with disease, whereas disease incidence in treated nursery was only 5.5% (Gill and Parvez, 1992). Therefore, efforts so far were given on seed treatment. There are few reports on the *in vitro* toxicity of fungicides to *F. moniliforme* (Garibaldi, 1985; Sasaki, 1987). Fungicides of organo-marcurial and benzimidazol groups have generally been reported to be effective against this disease (Ou, 1985). However, disease control at different locations varied with fungicides, their concentration, period of soaking and isolates of pathogen (Kauraw, 1981 andWada *et al.,* 1990).

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Furthermore, in Bangladesh, there are no registered fungicides as seed treatment for *F. moniliforme*. Keeping in view the importance of fungicides for disease control, studies were undertaken on the efficacy of fungitoxicants against bakanae disease to evaluate the *in vitro* efficacy of different fungicides.

METHODOLOGY

The experiment was conducted in the Plant Pathology Laboratory at Bangladesh Rice Research Institute (BRRI) and farmer's field. Efficacy of fungicides was assessed to control a) seed-borne infection, b) seedling infection in pot and c) seedling infection in seed-bed.

Seed-borne infection. About 12 kg seeds of BR1 were collected from bakanae infected fields. About 250 gm of seeds was taken in a cloth bag and 33 cloth bags were used. Eleven fungicides of different fungicidal groups were tested. Table 1 presents the details about the fungicides. Three different suspensions of each fungicide were prepared by adding 2.5, 3.0 and 3.5 gm commercial fungicide/L water for each kg seeds. The seeds were submerged under the fungicidal suspension for overnight (10-12 hrs). Afterward the excess suspension was drained out. After soaking for over night one part of fungicides treated seeds were washed with sterilized distilled water for 3-4 times and dried in shed. Other parts of treatedseeds, also dried in shed, were kept unwashed. Control treatment was provided by treating seed using sterilized distilled water only without fungicides. The treated seeds were tested by blotter method to detect *F. moniliforme*following international rules for testing seed health(ISTA, 2000). Number of germinated seeds on which *F. moniliforme* grew was recorded. For proper identification temporary slides were prepared from the fungal colony, observed under compound microscope and identified following the method described by Nath*et al.* (1970).

Seedling infection (in pot). The fungicides effective for controlling seed-borne infection of *F. moniliforme*were selected. Pots substratum were prepared by mixing soil, sand and well decomposed cow dung in the proportion of 2:1:1. The mixture was sterilized at 121°C temperature under 15 PSI. The pots (33 × 25 cm^2) were filled up with the sterilized soil. Sixteen pots were used. Fungicidal solutions of selected fungicides vizBavistin, SunphanateCarzed and Nativo were prepared separately by mixing of these fungicides @ 2.5 gm/L of water. Infected seeds of BR1 were treated by soaking the seeds in fungicidal solution for overnight (10-12 hrs). Treated seeds were sown on the pot @ 50 seeds/pot. The experiment was laid out in CRD with four replications. Number of germinated seeds and bakanae infected seedlings were recorded.

Seedling infection (in seed-bed). The promising fungicides further tested in seed-bed during Aus 2010 and 2011 seasons in farmer's field at Habiganj district. A seed-bed of 15 × 15 m in a medium low land was prepared. Naturally bakanae infected seeds of BR1 were treated with these fungicides separately by soaking the seeds in fungicides solution for overnight @ 2.5 gm/L. Seeds were dipped in fresh water considered as control. Then the suspension of fungicides and water was drained out and treated seeds were incubated for 48 hours for sprouting. Sprouted seeds were sown in the seed-bed separately. Sufficient water was applied to the growing seedlings in the seed-bed. After 15 days of seeding bakanae infected seedlings were counted and continued up to 25 days.

Data analyzed

Collected data were converted into percent germination and percent seed-borne fungus and seedling infection. Finally the data were analyzed by using statistical package programme (MSTAT-C). Duncan Multiple Range Test (DMRT) was used for comparison of treatment means.

Table 1. Fungicides used for seed treatment.

Chemical group	Active ingredient	Trade name	Doses (gm/L)* *cf/kg
Benzimidazole	Carbendazim	Bavistin 50WP	2.5, 3.0, 3.5
Carbamate	Thiophenate methyl	Sunphanate 70WP	2.5, 3.0, 3.5
Not found	Tricyclazole	Trigger 75WP	2.5, 3.0, 3.5
Mancozeb	Dithiocarbamate	Greenzeb	2.5, 3.0, 3.5
Dithiolane	Isoprothioline	Sunthio 40 EC	2.5, 3.0, 3.5
Dicarboximide	Iprodione	Rovral 50WP	2.5, 3.0, 3.5
Inorganic	Copper oxichloride	Gunner	2.5, 3.0, 3.5
Mixed	Mancozeb 63% + Carbendazim 12%	Carzeb 75 WP	2.5, 3.0, 3.5
Mixed	Tabuconazole 50% + Trifloxystrobin	Nativo 75 WP	2.5, 3.0, 3.5
Mixed	55% Metiram+ 5% Pyraclostrobin	Cabrio 60 WG	2.5, 3.0, 3.5

Mixed	2.5%	Fludioxonil	+	2.5%	Celest extra 5EC	2.5, 3.0, 3.5

*Commercial formulation.

RESULT

Effect on seed-borne *F. moniliforme* (without washing).Table 2 presents the results on the effect of fungicides on the incidence of *F. moniliforme*. The incidence of *F. moniliforme* with different doses such as 2.5, 3.0 and 3.5 gm/L differed significantly among the treatments. Seed treatment at 2.5 gm/L with Carzeb, Sunphanate, Nativo, Bavistin and Celest extra controlled cent percent infection of *F. moniliforme*. The incidence of the pathogen in Gunner and Rovral treated seed was 14 and 13.5% and did not vary statistically. Similarly, seed treated with Gunner and Rovral and Trigger and Sunthio showed identical results. On the other hand, only 1.25% seed yielded *F. moniliforme* when the seeds were treated with Greenzeb at 2.5 gm/L. The highest incidence (22.25%) was recorded in control treatment.

Incidence of *F. moniliforme* was reduced due to seed treatment with fungicides at 3 gm/L concentration as compared to the 2.5 gm/L. The highest 22.25% infection was found in control treatment. The incidence of Gunner, Sunthio and Rovral treated seeds were 8.00, 8.50, and 9.50% respectively. Seed treated with, Cabrio and Trigger showed similar results. Further increase of fungicidal dose (3.5 gm/L), decreased the incidence of *F. moniliforme.*

Table 2.Effect of fungicides on seed-borne infection of *Fusariummoniliforme* in rice seeds (without washing).

Chemical	% incidence at three doses of fungicides (gm/L)		
	2.50	3.00	3.50
Carzeb 75WP	0.00 e	0.00 d	0.00 g
Sunphanate 70WP	0.00 e	0.00 d	0.00 g
Nativo 75WG	0.00 e	0.00 d	0.00 g
Bavistin 50 WP	0.00 e	0.00 d	0.00 g
Celest Extra 050FS	0.00 e	0.00 d	0.00 g
Trigger 75WP	7.25 c	3.50 c	2.93 ef
Gunner	14.0 b	8.00 b	6.50 c
Sunthio 40EC	9.00 c	8.50 b	9.75 b
Cabrio 60WG	4.18 d	1.75 cd	1.50 fg
Rovral	13.50 b	9.50 b	5.00 d
Greenzeb	1.25 d	1.25 d	1.00 fg
Control	22.25 a	22.25 a	22.25 a

Means followed by same letter in a column did not differ at the 5% level of significance by DMRT.

Effect on seed-borne *F. moniliforme* (after washing).Table 3 presents the performance of the fungicides after washing of treated seeds. Different fungicides had significant effect on the incidence of *F. moniliforme*. Cent percent inhibition of the pathogen *F. moniliforme*was found when the seeds were treated with Bavistin at 2.5 gm/L followed by Sunphanate, Carzeb and Nativo. The incidence of the pathogen by Celest Extra, Trigger and Cabrio treated seeds were 6.75, 12.75 and 9.50% respectively. These were statistically significant. The incidence was identical in control, Rovral and Gunner treated seeds. At 3 gm/L, all the fungicides significantly reduced the incidence of *F. moniliforme*as compared with 2.5 gm/L. Gunner treated seed yielded 18.50% infection, which was similar to seed treated with Rovral. There was no difference between the treatment of Rovral and control. In further increase of the dose (3.5 gm/L), none of the fungicides completely eradicated the infection of the pathogen except Sunphanate. Sunphanate showed cent percent inhibition of *F. moniliforme* at 3.5 gm/L followed by Carzeb and Greenzeb. Although it's corresponding low dose did not show complete inhibition. The infection in Gunner and Rovral treated seeds did not differ statistically. Similarly, the performance of Trigger and Sunthio at dose 3.5 gm/L was identical.

Table 3.Effect of fungicides on seed-borne infection of *Fusariummoniliforme* in rice seeds (after washing).

Chemical	% incidence at three doses of fungicides (g/L)		
	2.50	3.00	3.50
Carzeb 75WP	3.75 ef	3.50 fg	1.25 e
Sunphanate 70WP	1.75 fg	1.25 g	0.00 e
Nativo 75WG	2.25 fg	2.25 fg	2.00 e
Bavistin 50WP	0.00 g	0.00 g	0.00 e
Celest Extra 050FS	6.75 d	8.75 de	5.50 d
Trigger 75WP	12.75 b	13.25 c	11.25 c
Gunner	25.50 a	18.50 b	16.00 b
Sunthio 40EC	12.00 bc	10.50 cde	9.50 c
Cabrio 60WP	9.50 c	6.50 ef	5.00 d
Rovral	24.50 a	22.50 ab	15.00 b
Greenzeb	5.50 de	4.25 fg	2.00 e
Control	26.50 a	26.50 a	26.50 a

Means followed by same letter in a column did not differ at the 5% level of significance by DMRT.

Effect of fungicides on seed germination.

Table 4 presents the effects of fungicides on seed germination. At 2.5 gm/L all fungicides significantly increased the germination percentage over control. The highest germination (89.50%) was found in Greenzeb treated seeds. These were statistically similar in seed germination for all other fungicidal treated seeds except Triger. In case of Triger, the germination was 68% and did not vary significantly with the germination of control treatment. The lowest germination (67.50%) was recorded in control. All fungicides also increased seed germination at the dose of 3 gm/L. However, at fungicidal dose of 3.50 gm/L, seed germination decreased in case of most of the fungicides.

Table 4.Effect of fungicides and their doses as seed treatment on seed germination.

Fungicide	% seed germination at different rates of fungicides (gm/L)		
	2.50	3.00	3.50
Carzeb 75WP	87.50 ab	79.00 b	71.00 de
Sunphanate 70WP	80.50 adc	80.50 ab	83.50 ab
Nativo 75WP	83.50 ab	83.00 ab	75.00 cd
Bavistin 75WP	85.00 ab	85.25 ab	84.75 ab
Celest Extra 050FS	83.00 ab	84.25 ab	82.75 abc
Trigger 75WP	68.00 d	78.25 b	70.00 de
Gunner	79.50 abc	80.00 b	72.50 de
Sunthio 40EC	72.50 cd	85.00 ab	81.50 abc
Cabrio 60WP	84.00 ab	81.50 ab	82.00 abc
Rovral	83.50 ab	83.00 ab	80.50 abc
Greenzeb	89.50 a	89.00 a	77.50 bc
Control	67.50 d	67.50 c	67.50 e

Means followed by same letters in a column did not differ at the 5% level of significance by DMRT.

Figure 1 shows the mean of the germination as influenced by different fungicides. Maximum 85% germination was found in Greenzeb and Bavistin treated seeds. The germination was 83, 82, 82 and 81% respectively in Cabrio, Rovral, Sunphanateand Nativo treated seeds respectively. Seed treated with Carzeb and Sunthio had 79 and 80% germination. Minimum 68% germination was recorded in control treatment.

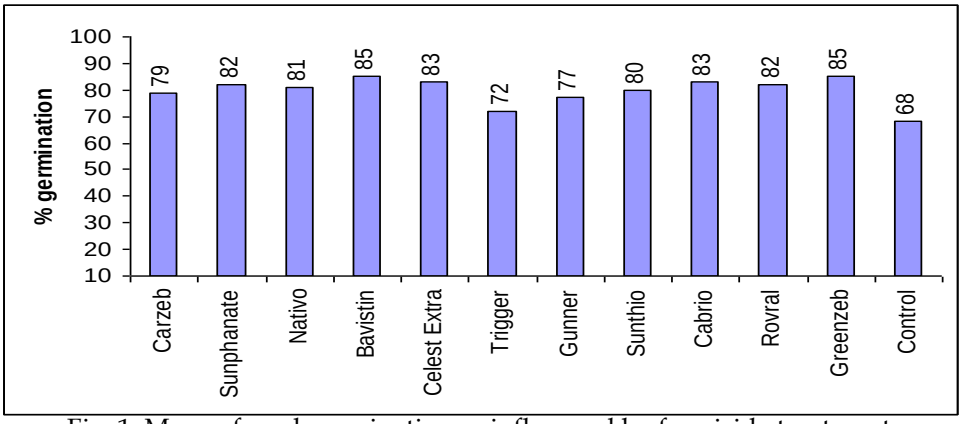

Fig. 1. Mean of seed germination as influenced by fungicide treatment.

Effect of promising fungicides on bakanae (Pot experiment). Table 5 presents the effect of selected fungicides on infected rice seeds tested on pot soil. Seed germination and incidence of bakanae infected seedlings at 10 and 20 days after seeding (DAS) were observed. The highest germination (85.83%) was found in Bavistin treated seeds followed by Sunphanate (83%) treated seed. The lowest germination (65.33%) was recorded from control treatment. There was 82.50 and 80.17% germination in Nativo and Carzed treated seeds. There was no infected seedling from treated seeds even at 20 DAS. However, in control treatment there was 31.5 and 42.36% bakanae infected seedlings at 10 and 20 DAS respectively.

Table 5.Effect of fungicides as seed treatment on the incidence of bakanae seedlings on pot soil.

Treatment	Germination (%)	% incidence of bakanae	
		Days after seeding (DAS)	
		10	20
Bavistin 50WP	85.83 a	0 b	0 b
Sunphanate 70WP	83.00 a	0 b	0 b
Carzeb 75WP	80.17 b	0 b	0 b
Nativo 75WG	82.50 b	0 b	0 b
Control	65.33 c	31.5 a	42.36 a

Means followed by same letter in a column do not differ at the 5% level of significance by DMRT.

Effect of promising fungicides on bakanae in seed-bed. The performance of selected fungicides for seed treatment was compared to untreated seeds in the seed-beds. The seeds were treated with the fungicides before sowing in the seed-bed. Table 6 presents the results of fungicidal treatment of rice seeds on bakanae incidence in seed-bed. All fungicides significantly reduced bakanae disease in seed-bed. In case of 2009, the lowest bakanae infected seedlings (0.67%) was found in the seed-bed, where Bavistin and Sunphanate treated seeds were sown. The incidence of bakanae was 1.3% and 1.0% in Carzeb and Nativo treated seed-bed. In 2010, again Bavistin and Sunphanate treated seed-bed, yielded significantly the lowest infection (1%). Carzeb treated seed-bed yielded 2% infection whereas it was 1.67% in Nativo treated seed. The highest bakanae infected seedlings (6%) was found in control seed-bed.

Table 6.Effect of fungicides as seed treatment on the incidence of bakanae disease in seed-bed.

Treatment	% incidence of bakanae infected seedling	
	2009	2010
Bavistin 50WP	0.67 b	1.00 b
Carzeb 75WP	1.33 b	2.00 b
Sunphanate 70WP	0.67 b	1.00 b
Nativo 75WG	1.00 b	1.67 b
Control	7.00 a	6.00 a

Means followed by same letter in a column, did not differ at the 5% level of significance by DMRT.

DISCUSSION

Bakanae disease of rice caused by *Fusariummoniliforme* occurs in almost all rice growing countries of the world including Bangladesh. The causal agent of the disease is seed-borne pathogens and can caused reduction in seed germination, attack mature plants and developing seeds (Khan *et al.,* 1990). The fungicides have great impact on the germination of seeds by suppressing the seed-borne pathogen and enhance the overall germination percentage. Thus, the impact of different fungicides on the inhibition of seed-borne *F. moniliforme* as well as seed germinationwas studied using standard blotter method. The results revealed a significant response in terms of inhibition of seed-borne *F. moniliforme*. Among the fungicides Bavistin, Sunphanate, Carzeb and Nativo completely eradicated the pathogen from the infected seeds at low (2.5 gm/L) dose. The other fungicides having higher concentrations also reduced the pathogen significantly. Effectiveness of fungicides against *F. moniliforme*has been suggested by many researchers (Haque*et al.,* 1980; Sarker, 1986; Bhalli*et al.,* 2001; Akter, 2001; Latif*et al.,* 2007; Shakahwat, 2009). They documented that seed treatment with Bavistin and Sunphanate consistently provided good protection against *F. moniliforme* and other fungi associated with rice seeds. The findings of Biswas and Das (2002) also strongly support the results of the study. They stated that Bavistin, Emisan-6 and Benomyl were the most inhibitory against *F. moniliforme.*

In Bangladesh, broadcasting of sprouted seeds in puddled seed-bed is a common practice for raising rice seedling. This practice, probably have an adverse effect on the efficacy of fungicides of treated seeds. Therefore, in present study, treated seeds were also subject to surface washing before planting for fungal recovery. It was observed that surface washing reduced the effectiveness of the fungicides to some extent. However, even after washing, in case ofBavistin treated seeds (seeds pre soaked in Bavistin solution), the efficacy was found to remain unchanged. This might be the efficacy of Bavistin on seed-borne inocula of *F. moniliforme* is comparatively quick, which might have killed the pathogen by over night soaking. These results are in agreement with the findings of Sunder *et al.* (1998); Latif*et al.* (2007). They reported that Carbendazim was best while the treated seeds were washed after treatment. Therefore, under such condition Bavistin could be used to get better disease control. Regarding germination, it was found to be better at 2.5 gm/L dose (89.9%) of Greenzeb. By increasing the fungicidal dose (3 gm/L) the germination percentage increased. Although further increase of dose most of the cases germination percentage decreased. The findings are in accordance with the result of Bhalli*et al.* (2001) and Gill *el al.* (1999). Bavistin andGreenzed exhibited best results with maximum (85%) mean germination followed by Celest Extra. The results were in accordance with those of Iqbal*et al.* (2013). Similar studies also carried out by Ilyas and Iftikhar (1997) with fifteen different fungicides.

CONCLUSIONS

Bakanae is one of the most destructive disease of rice in Bangladesh and the causal agent of the disease is seed borne. Seed treatment with Bavistin, Sunphanate, Nativo and Carzeb completely eradicate the pathogen from the seeds. These findings are important especially concerning a seed certification programme in which seed lot are certified on the basis of field infection. Finally, controlled experiments are needed to draw more general conclusion.

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