EVALUATION OF FUNGICIDES AGAINST *RHIZOCTONIA ORYZAE-SATIVAE*, THE CAUSAL FUNGUS OF AGGREGATE SHEATH SPOT OF RICE

S. B. Jahan[1], M. A. Ali[2], S. Alam[1], Z. R. Moni[3] and M. S. Mian[2]

[1]Department of Botany, Rajshahi University, Rajshahi, Email: shapnattc@yahoo.com; [2]Plant Pathology and [3]Plant Breeding Divisions, Bangladesh Rice Research Institute, Joydebpur, Gazipur-1701, Bangladesh

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ABSTRACT

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An *in-vitro* investigation was conducted to screen eight fungicides against *Rhizoctonia oryzae-sativae*, the causal fungus of aggregate sheath spot disease of rice. The fungicides were Amistar top 325 SC (Azoxystrobin), Carbendazim 50WC (Carbendazim), Differ 300EC (Difeconazole-propiconazole), Folicur 250EW (Tebuconazole), Monceren 250SC (Pencycuron), Mancodazim 63%+12% (Mancozeb-Carbendazim), Nativo 75WG (Trifloxystroin-Tebuconazole) and Propi 25EC (Propiconazole). Potato dextrose agar (PDA) was amended with the fungicides at 0.00, 0.25, 0.50, 1.00, 10.00 and 100.00 ppm. Amended PDA was poured into Petri dishes at 20 ml per dish and inoculated with mycelia blocks of *R. oryzae-sativae*. *Rhizoctonia oryzae-sativae* was found to be sensitive to all the fungicides tested. Carbendazim showed strong activity against the pathogen showing the lowest LD (lethal dose) 90 values (0.8 ppm) and LD 50 (0.1 ppm). Field trial was conducted to test the efficacy of all fungicides to control aggregate sheath spot of rice. Of them Carbendazim, Tebuconazole and Trifloxystroin-tebuconazole significantly reduced disease development and increased rice yield by 14.58, 11.88 and 9.79%, respectively.

Key words: *Rhizoctonia oryzae-sativae*, aggregate sheath spot, fungicides, rice

INTRODUCTION

Rice is the major food grain in Bangladesh. The requirement of rice is increasing every year in the country with the increasing population. In Bangladesh, rice yield per hectare is about 3.0 tons which is very low compared to global average (Anon. 2014). Low production of rice per unit area is attributed to various biotic and abiotic factors. Disease is one of the most important factors for low yield of rice. Among different diseases attacking rice, aggregate sheath spot caused by *Rhizoctonia oryzae-sativae* (teleomorph *Ceratobasidium oryzae-sativae*) is notable to cause considerable reduction in yield (Ou 1985). Aggregate sheath spot may cause 20% yield losses in Australia and 4 to 9% in Uruguay (Lanoiselet *et al*. 2005). Its incidence and severity began to increase with the introduction of semi-dwarf rice cultivars in different rice growing countries of the world (Gunnel and Webster 1984).

Different methods of plant disease control including chemical, physical, cultural measures are recommended to control aggregate sheath spot of rice. Globally chemical control represents the most widely used practice to reduce yield loss caused by *Rhizoctonia* spp. because no alternative satisfactory method is available. In California, Azoxystrobin has been registered for the control of rice aggregate sheath spot disease. In Australia, Pyraclostrobin tolclofos-methyl and Propiconazole were found effective fungicides for reducing mycelial growth of *R. oryzae-sativae in vitro* and disease severity under field conditions (Lanoiselet *et al*. 2005).

In Bangladesh, aggregate sheath spot was observed first time in the research farm of Bangladesh Rice Research Institute (BRRI) in both local and modern cultivars (Shahjahan *et al*. 1988). Presently, the disease is widely distributed throughout the country (Ali and Archer 2004). Sharma (2002) found that the growth of *R. oryzae-sativae* was completely inhibited at 1 ppm *in vitro* due to amendment of medium with fungicides.

The present piece of research was conducted to evaluate efficacy of eight fungicides *in-vitro* and under field condition to control *R. oryzae-sativae* isolated from aggregate sheath spot infected rice sheaths.

MATERIALS AND METHODS

In-vitro evaluation of fungicides

Eight fungicides namely Amistar top 325SC (Azoxystrobin), Carbendazim 50WC (Carbendazim), Differ 300EC (Difeconazole-propiconazole), Folicur 250EW (Tebuconazole), Monceren 250SC (Pencycuron), Mancodazim 63%+12% (Mancozeb-Carbendazim), Nativo 75WG (Trifloxystroin-

Tebuconazole) and Propi 25EC (Propiconazole) were tested in the experiment. Each of the fungicides was tested @ 0.00, 0.25, 0.50, 1.00, 10.00 and 100.00 ppm. Independent experiment was conducted to test each fungicide.

The test fungus, *R. oryzae-sativae* (Isolate MY-1) was obtained from Plant Pathology Division, BRRI, Gazipur. Poison food technique was followed in the experiment using potato dextrose agar (PDA) as the basal medium (Dhingra and Sinclair 1985, Ali and Archer 2003). Stock solution of 1000 ppm concentration was prepared in distilled water. Ingredients of PDA (Dhingra and Sinclair 1985) were mixed in 500 ml distilled water in conical flasks and appropriate quantity of stock solution was added to each flask to have required concentrations of individual fungicides. The mixture was thoroughly mixed and autoclaved at 120C under 0.80 kg/cm^2 pressure for 15 min. The amended PDA was dispensed into glass Petri dishes (90 mm dia) at 20 ml per dish. Plates received PDA without amendment served as control.

Five millimeter diameter mycelium blocks were cut from actively growing zones of 3-day-old *R. oryzae-sativae* culture which were used as inocula. The inocula were placed at the center of PDA plate keeping the up side down. The inoculated plates were arranged on the laboratory desks of Plant Pathology Division, BRRI, Gazipur. Three plates (replications) were used for each treatment. The plates were incubated at room temperature (30±2C). Diameter of colony of the fungus in plates containing amended PDA was measured when the mycelia in control plates reached the rim of the Petri dishes. Percentage of mycelium growth inhibition was computed based on colony diameter in control Petri dishes using the following formula:
Growth inhibition (%) =(C-T)/C X 100, Where, C= Growth of fungus in control plate, T = Growth of fungus in treated plates.

Field evaluation of fungicides

Based on findings of the *in vitro* test, five fungicides namely Carbendazim 50WC, Differ 300EC, Folicur 250EW, Nativo 75WG and Propiconazole 25EC were selected for their field evaluation against aggregate sheath spot during T. Aus seasons of 2012 and 2013 in the Research Farm of BRRI, Gazipur. BRRI dhan 48 was used in the experiment. Carbendazim, Propiconazole and Folicur were suspended in water at 0.90%. Suspension of Differ and Nativo was prepared in water at 0.18 and 0.036%, respectively. The experiment was laid out in randomized complete block design with three replications and 3m x 2m unit plot and plot to plot distance was 50cm.

Seeds of variety BRRI dhan 48 were treated with hot water at 52C for 20 min and soaked in plain water for 24 hours for sprouting. Sprouted seeds were sown in tray to raise seedlings. Thirty days old seedlings were transplanted in the experimental plot at 3-4 seedlings per hill maintaining 15 cm x 20 cm spacing. Pure culture of *R. oryzae-sativae* (Isolate MY-1) was multiplied on PDA plate and incubated at ambient temperature. After 7 days of incubation the culture in a plate was divided into 8 equal sections, which were used as inocula to inoculate test plants according to Lanoiselet *et al.* (2001). For inoculation, each portion of the PDA culture was inserted at the base of each hill at maximum tillering stage. Nine hills in the middle of each unit plot were inoculated. The fungicides were suspended in water at required concentration and sprayed twice at 7 days starting from 15 days after inoculation. Standard agronomic practices were followed to grow the crop to maintain normal growth of plants.

Data on infected and healthy tillers, plant height and top most lesion height were recorded from which incidence, relative lesion height (RLH) and severity was computed according to Ahn *et al*. (1986) and Yoshimura (1954) as described below:

Relative lesion height (RLH) (%) = (Lesion height/Plant height) X 100.

Incidence (%) = Number of infected plant / Number of total plant checked X 100.

Severity (%) = $(3N_1+ 2N_2 + N_3 + 0N_4)/3N)$ X 100, where N_1= number of tillers with all four uppermost infected sheaths; N_2=number of tillers with the three uppermost infected sheaths; N_3=number of tillers with the two uppermost infected sheaths and N_4=number of tillers with the four uppermost sheaths disease free. The crops were harvested at 80% maturity. After threshing, the grains were sun dried to have 14% moisture content and grain yield per nine selected hills was recorded. The grain was expressed in kilogram per hectare. Sterility and thousand grain weight were also recorded.

Data analysis

Standard errors of means of three replications (mycelial inhibition) were calculated using computer software Stata (version 12). The LD_{50} and LD_{90} values were calculated using the software Origin 7.0. Data of field trial were analyzed using MSTAT-C computer program and mean separation was performed using least significant difference (LSD) test.

RESULTS AND DISCUSSION

In-vitro evaluation of fungicides

All fungicides at 0.10, 0.25, 0.50, 1.00, 10.00 and 100.00 ppm concentrations significantly (P=0.05) inhibited radial colony diameter of *R. oryzae-sativae* on amended PDA compared to control. The effectiveness of every fungicide to inhibit colony growth was corroborated with its dosage showing the highest inhibition at the highest concentration and the lowest at the lowest concentration. Differences in growth inhibition at different concentrations of Carbendazim, Differ, Folicur, Mancodazim, Nativo and Propiconazole were significant. The growth inhibition was 100% at 100, 10 and 1 ppm of Carbendazim, 100 and 10 ppm of Folicur and 100 ppm of Differ, Mancodazim, Nativo and Propiconazole. Gowth inhibition of 50.4-68.3, 32.9-98-4, 12.7-75.0, 13.5-88.9, 28.6-98.2 and 27.8-921% was obtained with Carbendazim, Differ, Folicur, Mancodazim, Nativo and Propiconazole, respectively. The growth inhibition ranged 12.3-54.4 and 16.7-94.1% at 0.1-100.00 ppm of Amistar Top and Monceren, respectively (Table 1).

Carbendazim effectively inhibited the fungal growth more than 50% at 0.1 ppm. It means the LD 50 (lethal dose 50) of the fungicide was 0.01 ppm. LD 50 of Nativo and Propiconazole was 0.40 ppm and that of Folicur was 0.50 ppm. Similarly, LD 90 of Carbendazim was the lowest at 0.8 ppm followed by Folicur (0.9 ppm), Nativo (5 ppm) and Propiconazole (8 ppm). LD 50 of Monceren was 14 ppm followed by Mancodazim (2.1 ppm) and Differ (1.1 ppm). LD 90 of Amistar top was 15 ppm and that of Differ was 13 ppm (Fig. 1 and 2).

Field evaluation of fungicides

Results on the efficacy of five fungicides tested under field conditions during T. Aus season of 2012 and 2013 to control aggregate leaf spot of rice are shown in Tables 2 and 3.

In both years of experimentation, significantly the highest relative lesion height (RLH), disease incidence (DI), disease severity (DS) and grain sterility, and the lowest thousand grain weight (TGW) and grain yield were recorded from diseased control. On the contrary, the lowest disease related parameters and the highest yield related parameters were recorded from healthy control.

Significant decrease in RLH, DI, DS and grain sterility, and increase in TGW over diseased control was achieved with the application of Carbendazim, Differ, Folicur and Nativo. The most effective one was Carbendazim followed by Folicur and Nativo to decrease disease related parameters and to increase grain yield significantly compared to control. Efficacy of Folicur and Nativo was statistically similar and significantly lower compared to Carbendazim. Effectiveness of Differ and Propiconazole was almost similar. Propiconazole was noted as the least effective fungicide to control aggregate leaf spot. In general, DI, RLH and DS were appreciably lower under all treatments in 2013 than 2012 (Tables 2 and 3).

In the present investigation, *R. oryzae sativae* reduced yield by 0.73 t/ha that counts to 13.44% in diseased control compared to healthy control during 2012. Application of Carbendazim, Folicur and Nativo increased rice yield by 10.63, 8.51 and 6.38%, respectively over control. In 2013, rice yield was also significantly affected by aggregate sheath spot disease. *Rhizoctonia oryzae sativae* reduced yield of BRRI dhan48 by 0.83 t/ha (14.74%) over healthy control. Yield recovery in Differ and Propiconazole treatments over disease control were not significantly different. In contrast, Carbendazim, Folicur and Nativo increased yield by 14.58, 11.88 and 9.79%, respectively over control. The disease incidence, RLH and disease severity were lower in 2013 compared to 2012.

Results of both *in vitro* and *in vivo* screening of fungicides against *R. oryzae sativae* and aggregate sheath spot disease caused by the fungus reveal that Carbendazim, Folicur and Nativo are most effective for controlling the disease. Effectiveness of fungicide tested in the present investigation to inhibit mycelial growth increased with increasing concentration from 0.1 to 100 ppm. Similar results have been reported by Lanoiselet *et al.* (2005). They found significant reduction of *R. oryzae-sativae* growth when concentration increased from 0.1 to10 µg/ml. Only Carbendazim inhibited 100% mycelial growth at 1.0 ppm and higher concentrations used. Similar result with Carbendazim was also reported by Sharma (2002) based on an *in vitro*. Amister top (Azoxystrobin), Monceren (Pencycuron) and Mancodazim were poorly effective in vitro and excluded from field test. Poor efficacy of Azoxystrobin on mycelial growth of *R. oryzae-sativae* and *R. solani* has also been reported by Lanoiselet *et al.* (2005) and Ali and Archer (2003). In the present experiment, Differ and Propiconazole showed better inhibition *in vitro* but did not perform well *in vivo* in field test. The findings are in agreement the findings of Carling *et al.* (1990) and Martin *et al.* (1984a, 1984b).

Differ and Propiconazole though decreased disease severity over control but yield was not increase accordingly. Similar result was also found by Lanoiselet *et al.* (2005). The maximum yield loss caused by the disease was 14.74% (on BRRI dhan48 in 2013) that relates with the findings of Lanoiselet *et al.* (2005) who found 20.30% yield loss in Australia. Based on findings of the present investigation it may be concluded that aggregate sheath spot disease causes considerable yield loss and Carbendazim, Folicur or Nativo may be recommended to control the disease and to save yield loss. However, before final recommendation benefit cost ration need to be determined.

Table 1. Effect of different concentrations of eight fungicides on *in vitro* mycelial growth of *R. oryzae-sativae*

Fungicide	Radial colony diameter (mm) at different concentrations (ppm)						
	0.00 (Control)	0.10	0.25	0.50	1.00	10.00	100.00
Amistar Top 325 SC	84.00	70.00[a] (16.70)[b]	69.33 (17.50)	68.67 (18.30)	25.67 (69.40)	21.67 (74.20)	5.00 (94.10)
Carbendazim 50WC	84.00	41.67 (50.40)	31.67 (62.30)	26.67 (68.30)	0.00 (100.0)	0.00 (100.00)	0.00 (100.00)
Differ 300EC	84.00	73.33 (12.70)	60.33 (28.20)	46.33 (44.90)	43.33 (48.40)	21.00 (75.00)	0.00 (100.00)
Folicur 250EW	84.00	56.33 (32.90)	52.00 (38.10)	44.67 (46.80)	1.33 (98.40)	0.00 (100.00)	0.00 (100.00)
Mancodazim 63%+12%	84.00	72.67 (13.50)	72.00 (14.30)	68.67 (18.30)	59.33 (29.40)	9.33 (88.90)	0.00 (100.00)
Monceren 250SC	84.00	73.67 (12.30)	72.67 (13.50)	72.33 (13.90)	57.00 (32.10)	41.67 (50.40)	38.33 (54.4)
Nativo 75WG	84.00	60.00 (28.60)	54.00 (35.70)	32.67 (61.10)	25.67 (69.40)	1.50 (98.20)	0.00 (100.00)
Propiconazole 25EC	84.00	60.67 (27.80)	59.00 (29.80)	33.67 (59.90)	26.00 (69.10)	6.67 (92.10)	0.00 (100.00)

[a]LSD (0.05) computed to compare among means of different treatments except control is 4.07, and LSD computed to compare all means with the means of control is 3.73.
[b]Figures within parenthesis are % inhibition in radial colony diameter.

Table 2. Evaluation of fungicides against aggregate sheath spot disease of rice during T.Aus 2012

Treatment	Relative lesion height (RLH %)	Disease index (DI%)	Disease Severity (DS%)	Thousand grain weight (g)	Sterility (%)	Yield (t/h)
Carbendazim	10.92 D	38.89E	45.40D (32.15)*	22.26 AB	21.02 D	5.2 B (10.64)**
Differ	27.39 B	62.22B	59.57B (10.97)	21.52 CD	27.31 AB	4.8 D (2.13)
Folicur	15.01 C	52.27D	51.21C (23.46)	21.92 BC	22.61 CD	5.1 BC (8.51)
Nativo	17.15 C	55.56C	53.61C (19.88)	21.85 BCD	25.58 BC	5.0 C (6.38)
Propiconazole	28.14 B	64.44B	62.24B (6.98)	21.24 DE	27.77 AB	4.7 D (0.00)
Diseased Control	31.08 A	67.78A	66.91A	20.80 E	29.76 A	4.7 D
Healthy Control	7.82 E	6.67F	18.68E	22.58 A	20.53 D	5.43 A

In a column, means followed by the same letter are not significantly different (P= 0.05).
Values within parentheses are *% reduction in disease severity and **% increase in yield over diseased control.

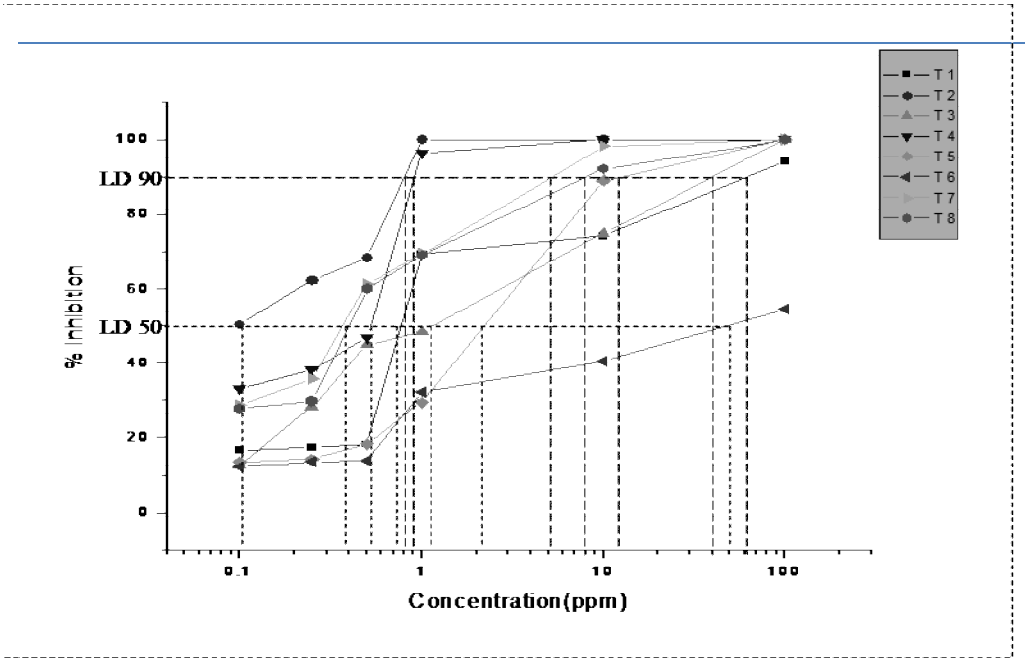

Figure 1. LD $_{50}$ and LD $_{90}$ values of different fungicides on growth inhibition of *R. oryzae-sativae* [T_1= Amister Top, T_2= Carbendazim, T_3= Differ, T_4= Folicur, T_5= Mancodazim, T_6= Monceren, T_7= Nativo and T_8= Propi]

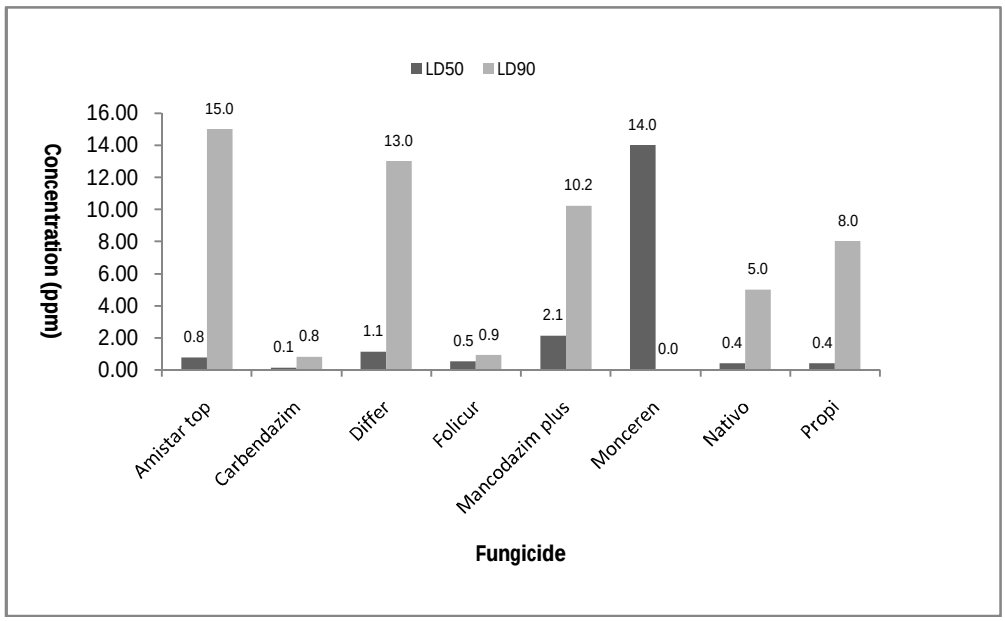

Figure 2. Values of LD 50 and LD 90 of different fungicides tested *in vitro* against mycelial growth of *R. oryzae-sativae*

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Table 3. Evaluation of fungicides against aggregate sheath spot disease of rice during T.Aus 2013

Treatment	Relative lesion height (RLH %)	Disease index (DI%)	Disease Severity (DS%)	Thousand grain weight (g)	Sterility (%)	Yield (t/h)
Carbendazim	6.93 E	12.22 D	40.79 E (38.14)	22.44 B	20.87 E	5.50 AB (14.58)
Differ	18.63 C	53.33 B	55.53 B (15.79)	21.80 D	26.01 BC	4.90 D (2.08)
Folicur	12.69 D	34.44 C	51.11 C (22.49)	22.17 C	22.40 DE	5.37 BC (11.88)
Nativo	14.12 D	37.78 C	47.29D (28.28)	22.09 C	23.56 CD	5.27 C (9.79)
Propiconazole	27.74 B	58.89 A	58.17 B (11.78)	21.37 E	26.50 AB	4.83 D (0.62)
Diseased Control	30.98 A	61.11 A	65.94 A	21.02 F	28.95 A	4.80 D
Healthy Control	5.48 F	4.44 E	14.50 F	22.80 A	18.12 F	5.63 A

In a column, means followed by the same letter(s) are not significantly different (P=0.05).
Values within parentheses are percent *% reduction in disease severity and ** Perent increase in yield over control.

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