Plant Pathology Journal

ISSN 1812-5387

science alert

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Pakistan Journal of Plant Pathology, Vol. 1 (1): 4-7, 2002

Effect of Fungicides Against *Bipolaris oryzae* of Rice Under *In vitro* Condition

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Abstract: Twelve seed samples of rice were tested and all were found infected by *Bipolaris oryzae* the cause of brown spot disease. Highest (5.5%) and lowest (1.5%) incidence was found in sample of Bhabokhali and Mahozompur, respectively. Four fungicides viz. Bavistin, Hinosan, Tilt 250 EC and Dithane M-45 were evaluated against *Bipolaris oryzae*. Dithane M-45 was the best with 100% reduction of the prevalence of the pathogen and inhibited the mycelial growth at 0.3% of the seed weight as seed treatments and 500 ppm as mycelial growth inhibition test followed by Tilt 250 EC, Hinosan and Bavistin. All test fungicides were effective against *Bipolaris oryzae* at higher concentration.

Key words: Fungicides, *Bipolaris oryzae*, rice, *In vitro* condition

Introduction

Rice is the staple food of Bangladesh. It covers about 75% of the total cropped area in the country (BBS, 1998). The average world yield of rice is 3.75 tons ha^{-1}, but the average yield of rice in our country is only 1.87 tons ha^{-1} (BBS, 1999). So the average per hectare production of rice in Bangladesh is extremely low compare to other rice growing countries of the world. There are many constraints responsible for low yield of rice in Bangladesh. Among the constraints, diseases are considered the most important. About 31 diseases of rice are reported to exist in Bangladesh and brown spot is one of the major diseases of rice (Miah *et al.*, 1985). In susceptible cultivars, brown spot disease may cause 5-10% yield loss (Miah, 1985). In severe infection, the yield loss due to *Drechslera oryzae* has been recorded as much as 30-40% (Aluko, 1975). *Bipolaris oryzae* is a seed borne pathogen and deteriorates the seeds in storage by reducing germination and affecting seed quality (Christensen and Kaufmann, 1965). It also causes disease to the emerged rice seedlings (Mia and Mathur, 1983).

Various methods have been practiced to control this pathogen. Seed treatment with fungicides reduced seed borne infection (Rao and Ranganathaiah, 1988) by *Bipolaris oryzae* and higher seedling vigour (Geetha and Sivaprakasam, 1993). Fungicides successfully inhibited the growth of *Bipolaris oryzae In vitro* (Sisterna and Ronco, 1994). The foliar application of fungicides have been successfully controlled brown spot disease (Singh *et al.*, 1985). In view of the above facts, this study was undertaken in laboratory conditions to determine comparative efficacy of different fungicides and their concentrations in controlling *Bipolaris oryzae* the cause of brown spot disease of rice.

Materials and Methods

The experiments were conducted in the Laboratory of Plant Pathology Department and Seed Pathology Laboratory (SPL), Bangladesh Agricultural University (BAU), Mymensingh during June to December, 2000. Twelve seed samples of rice variety BR 11 (Mukta) were collected from 12 villages of Mymensingh district, Bangladesh. Two hundred seeds were randomly collected from each sample. The seeds were plated on water soaked three layered blotter papers in plastic petridishes. In each petridish, 25 seeds were plated at equal distance. All these petriplates were incubated at 22 ± 1°C under 12 hours cycle of alternate near ultra violet light and darkness (ISTA, 1976). After 10 days of incubation, petriplates were observed under stereo-binocular microscope for the presence of *Bipolaris oryzae*. Temporary slides were prepared from the fungal colony of *Bipolaris oryzae* and examined under compound microscope. The samples with highest incidence of *Bipolaris oryzae* were selected for seed treatment. The fungal colonies were transferred to PDA in glass petridishes for purification. The purified cultures were kept in a refrigerator for subsequent uses.

The experiment was laid out in a completely randomized design (CRD) with 13 treatments (including control). The seed treating fungicides (treatments) were Bavistin (carbendazim), Hinosan (edifenphos), Dithane M-45 (mancozeb) and Tilt 250 EC (propiconazole). Every fungicide was tested at three concentrations such as, 0.20, 0.25 and 0.30% of the seed weight. Each fungicide was taken in a 250 ml conical flask containing 100 of seeds. The flasks were shaken for 15 minutes and then fungicide solutions were drained off and seeds were placed on moist filter paper in petridishes. Two hundred highest *Bipolaris oryzae* infected seeds from each treatment were plated and incubated at 25 ± 1°C for 10 days under 12 hr alternate cycle of near ultra violet light and darkness.

Seedling vigour test by paper roll method was done. In this method each fungicide was tested at three conc. such as 0.20, 0.25 and 0.30% of seed weight and one untreated control. Two hundred seeds for each treatment were tested by using paper towel method (Singh and Rao, 1977). Fifty seeds were placed in rows on two layered moistened paper towels and then the towels were rolled and closed both ends by using rubber bands and placed it in a up right position for 5-7 days at 30 ± 2°C and in light regime of 12 hours each day. After 7 days of setting of experiment the germination was counted. For determination of seedling vigour 10 seedlings were randomly selected from each towel band and their individual shoot and root length were measured. The vigour of the seedlings were determined by following the formula of Geetha and Sivaprakasam (1993) where:

VI = Germination % x root length (mean of 10 seedlings in cm)
VI = Germination % x shoot length (mean of 10 seedlings in cm)

Mycelial growth inhibition test was done by:

Poison food method:

- Fifty ml distilled water was taken into a conical flask (250 ml) and required amount of fungicides were mixed (e.g. 100 ml 500 ppm Bavistin solution = 100 mg, Bavistin). After mixing of fungicides, 50 ml of double quantity PDA medium was poured into the conical flask and then the poisoned PDA was poured in sterilized glass petridish. For control plate, only water was used. Each treatment was replicated thrice. Same procedure was followed for the other fungicides and their concentrations.
- The effect of fungicides on the growth of the fungus on agar medium was studied at 50, 100, 250 and 500 ppm concentration. The mycelial discs of the fungus (7 mm diameter) from actively growing culture were placed on the middle of the plate and incubated at 27 ± 1°C (Rao and Lalithakumari, 1987). The diameter of mycelial growth of the fungus was measured after 7 days of the incubation and growth inhibition was calculated.

Cup method: PDA plates were prepared and after solidification of the medium 3 cups of 7 mm diameter were made at three

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equidistant places by disc cutter. 50, 100, 250 and 500 ppm concentrations were also used in this method. Three drops of fungicides were put into each cup and the plates were stored in a refrigerator overnight. In the following day, fungal block (7 days old culture) containing culture medium was placed in cups. For control treatment, only water was used instead of fungicides. The plates were then placed at 26 ± 2^{0}C for 5 days. After 5 days data were collected as that of poison food method.

Recorded data were analyzed by Duncan's multiple range test (DMRT) to find out the differences of controlling efficacy of selected fungicides on *Bipolaris oryzae*.

Results and Discussion

Out of 12 seed samples tested all were infected with *Bipolaris oryzae* (Table 1). The percent infection was highest (5.5%) in sample no. 2 followed by sample no. 5 (4.5%). Percent infection was lowest (1.5%) in sample No. 12. All the samples were significantly different in respect of germination. Most of the seed samples showed germination below 80%.

The results of seed treatments with fungicides against *Bipolaris oryzae* in blotter are shown in Table 2. Fungicides increased percent seed germination. The highest germination was counted 74.5 % when the seeds were treated with Dithane M-45 at concentration 0.3% and followed by Tilt 250 EC at concentration 0.3. The lowest germination was 67.0% when the seeds were treated with Hinosan at 0.2%. All the test fungicides and their different concentrations significantly reduced the fungal population. *Bipolaris oryzae*, in seed was best controlled by Dithane M-45. Dithane M-45 controlled the pathogen 100% at concentration 0.3 and 81.8 % at concentration 0.25%. The second best was Tilt 250 EC which controlled the pathogen 90.9% of concentration 0.3% and followed by Bavistin and Hinosan. The lowest control was 27.3% with Hinosan at concentration 0.20%.

Table 1: Seed germination and percent incidence of *Bipolaris oryzae* in collected rice seed samples of BR11 (Blotter incubation test)

Samples	% Germination	Percent seed-borne infection of *Bipolaris oryzae*
1 = Bhabokhali	79.0ab	3.0
2 = Bhavkhali	68.5c	5.5
3 = Boira	80.5ab	2.5
4 = Dhigerkand	74.5a-c	3.0
5 = Barobillar par	70.0a-c	4.5
6 = Doribhavkhali	76.0a-c	2.5
7 = Raghobpur	72.0bc	3.0
8 = Narianpur	76.5a-c	2.5
9 = Shutiakhali	80.0ab	3.0
10 = Beltoli	78.0ab	3.5
11 = Vijoynagor	79.0ab	3.0
12 = Mahozompur	81.5a	1.5
LSD (p = 0.05)	1.83	NS

Figures in a column with common letter(s) do not differ significantly at 5% level. NS = Non significant

All the fungicides and their different concentrations were significantly different in respect of root length, shoot length and vigour index (Table 3). Highest root length (9.96 cm) was found when the seeds were treated with Dithane M-45 (0.3%) and the lowest root length (7.14 cm) was recorded in case of Hinosan (0.2%). The highest shoot length (5.04 cm) was also recorded when the seeds were treated with Dithane M-45 (0.3%) and the lowest shoot length (4.12 cm) was recorded with Hinosan (0.2%). Vigour index (based on root length) was highest (822.3) in Dithane M-45 (0.3%) followed by Tilt (0.3%) while the lowest index (488.6) was recorded in Hinosan (0.2%). Under vigour index (Based on shoot length), highest count (416.2) was observed when the seeds were treated with Dithane M-45 at 0.3% followed by Tilt 250 EC at 0.3%. The lowest count (283.9) was observed under the treatment Hinosan (0.2%).

All the fungicides and their different concentrations significantly inhibited the mycelial growth of *Bipolaris oryzae* (Table 4). Dithane M-45 showed the greatest efficacy on *Bipolaris oryzae* with the greatest effect at 500 ppm. 100 % of inhibition was obtained with Dithane M-45 at 500 ppm. Tilt 250 EC inhibited 95.58% mycelial growth at concentration of 500 ppm. The lowest inhibition (1.99%) was observed with 50 ppm of Bavistin. Fungicides and their concentrations were significantly inhibited the mycelial growth of *Bipolaris oryzae* (Table 5). Dithane M-45 500 ppm showed the greatest efficacy of 62.93 % inhibition. The second best efficacy (54.67) was observed with Tilt 250 EC at the concentration 500 ppm. The lowest inhibition of growth (11.2%) was observed with Bavistin at 50 ppm.

The efficacy tests of fungicides were done by seed treatment and mycelial growth inhibition test under laboratory conditions. All the test samples were found to be infected (1.5-5.5%) insignificantly with *Bipolaris oryzae* and germinability of most of the seed samples were below 80 % which is lower than the recommended national germination standard. These results agreed with the findings of Islam (1997). The lower range of infection was also supported by Fakir and Ahmed (1974). In their report 4.3 %

Table 2: Efficacy of fungicides on seed germination and controlling seed-borne *Bipolaris oryzae* of rice (BR11) following blotter incubation test

Treatments	Concentrations(%)	% Germination	Total fungal population (%)	Fungal reduction (%)
Control	0.00	66.5	5.5 a	-
Bavistin	0.20	67.0	3.0	45.5
	0.25	67.5	3.0a-c	45.5
	0.30	69.5	2.0b-d	63.6
Hinosan	0.20	67.0	4.0ab	27.3
	0.25	68.5	2.5b-d	54.5
	0.30	69.5	2.0b-d	63.6
Tilt 250 EC	0.20	68.0	3.0a-c	45.6
	0.25	70.5	2.0b-d	63.6
	0.30	720	0.5cd	90.6
Dithane M-45	0.20	69.5	1.5b-d	72.7
	0.25	71.0	1.0cd	81.8
	0.30	74.5	0.0d	100.0
LSD (p =0.05)		NS	0.574	

Table 3: Effect of treating seeds with fungicides on germination, root length, shoot length and vigour indices

Treatments	Concentration(%)	% Germination	Root length (cm)	Shoot length (cm)	Vigour index based on root length and percent germination
Control	0.00	69.0	6.94e	3.73f	471.8g
Bavistin	0.20	73.5	8.04e	4.65d	590.8f
	0.25	74.5	8.52c	4.94ab	634.6d-f
	0.30	78.0	8.65c	4.85c	674.8b-d
Hinosan	0.20	69.0	7.14e	4.12e	488.6g
	0.25	71.0	7.15e	4.17e	507.6g
	0.30	74.5	7.72d	4.29d	600.1ef
Tilt 250 EC	0.20	76.5	8.59c	4.70cd	657.5cde
	0.25	78.5	8.43c	4.70cd	661.5cde
	0.30	80.5	9.14b	4.79cd	735.6b
Dithane M-45	0.20	78.0	8.45c	4.65d	659.6cde
	0.25	81.5	8.78bc	4.84bc	715.6bc
	0.30	82.5	9.96a	5.04a	822.3a
LSD (p =0.01)		NS	0.367	0.17	59.22

Figures in a column with common letter(s) do not differ significantly at 1% level. NS = Not significant

Table 4: Effect of fungicides on inhibiting the mycelial growth of *Bipolaris oryzae* (poison food method)

Treatments	Concentrations (ppm)	Mean inhibition of growth (cm)	Mean percent inhibition of growth
Control	0	4.53a	-
Bavistin	50	4.44a	1.99
	100	4.23b	6.62
	250	4.03c	11.04
	500	3.25d	28.26
Hinosan	50	2.57e	43.27
	100	2.05f	54.75
	250	1.63g	64.02
	500	1.45g	67.99
Tilt 250 EC	50	2.59e	42.83
	100	1.55g	65.78
	250	0.78i	82.78
	500	0.20k	95.58
Dithane M-45	50	1.54g	66.00
	100	0.99h	78.15
	250	0.53j	88.30
	500	0.00l	100.00
LSD (p =0.01)		0.172	

Figures in a column with common letter(s) do not differ significantly at 1% level.

Table 5: Effect of fungicides on inhibiting the mycelial growth of *Bipolaris oryzae* (cup method)

Treatments	Concentrations (ppm)	Mean inhibition of growth (cm)	Mean percent inhibition of growth
Control	0	3.75a	-
Bavistin	50	3.33b	11.20
	100	3.17c	15.47
	250	2.88e	23.20
	500	2.70g	28.00
Hinosan	50	3.27bc	12.80
	100	3.03d	19.20
	250	2.74fg	26.93
	500	2.64g	29.60
Tilt 250 EC	50	3.25bc	13.33
	100	2.86ef	23.73
	250	2.45h	34.67
	500	1.70j	54.67
Dithane M-45	50	3.23bc	13.87
	100	2.76efg	26.40
	250	2.28i	39.20
	500	1.39k	62.93
LSD (p =0.01)		0.121	

Figures in a column with common letter(s) do not differ significantly at 1% level.

Helmithosporium oryzae infection was recorded in the collected samples from Bangladesh Agricultural University (BAU) farm during 1970.

In controlling seed-borne *Bipolaris oryzae* in seed with fungicides by blotter incubation test, Dithane M-45 and Tilt 250 EC were found effective at 0.3%. *Bipolaris oryzae* was best controlled by Dithane M-45 at 0.3%. Dithane M-45 and Tilt 250 EC inhibited the growth of the pathogen by 100 and 90.9 % over control at 0.3% respectively. These findings agree with that of Islam *et al.* (1992). Dharam *et al.* (1970) also reported the complete elimination of *Drechslera oryzae* by treating seeds with Dithane M-45 at 0.3 % of the seed weight.

In case of seed germination, root-shoot elongation and seedling vigour, Dithane M-45 and Tilt 250 EC were effective. Dithane M-45 was showed the best effect at 0.3% concentration. Tilt 250 EC (0.3%) ranked next in effective on germination, root-shoot elongation and vigorous indices.

The result agreed with the findings of Geetha and Sivaprakasam (1993). They reported that the treated seeds showed higher seedling vigour with 0.4% Mancozeb than untreated. Misra and Singh (1972) obtained better performance on germination percent and growth of seedling when the seeds were treated with fungicides of different concentrations. The results of mycelial growth inhibition test indicate that all the test fungicides possess inhibitory effect. However, considering their efficacy, Dithane M-45 was noted as the suitable fungicide followed by Tilt 250 EC, Hinosan and Bavistin against *Bipolaris oryzae*. Excellent performance of Dithane M-45 in controlling the mycelial growth of *Bipolaris oryzae* was exhibited at 500 ppm where growth of the fungus totally inhibited. Sisterna and Ronco (1994) reported Dithane M-45 as the best fungicide against *Bipolaris oryzae*, with the greatest effect at 500 ppm. In this concentration, all the fungicides significantly reduced the mycelial growth. However, Tilt 250 EC, Bavistin and Hinosan also showed inhibitory effect on the growth of *Bipolaris oryzae*. Viswanathan and Narayanasamy (1992) also reported significant result with the same fungicide at 1000 and 2000 ppm. The lowest effect of Bavistin and Hinosan supported by Swaminathan and Lalithakumari (1982).

So, it has been clearly observed that all test fungicides were effective against *Bipolaris oryzae* at higher concentration. Among them, Dithane M-45 was the best fungicide.

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