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Effect of Plant Extracts Against *Bipolaris oryzae* of Rice under *In vitro* Conditions

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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 plant extracts viz. biskatali (*Polygonum hydropiper*), onion (*Allium cepa*), garlic (*Allium sativum*) and neem (*Azadirachta indica*) were evaluated against *Bipolaris oryzae*. Among the plant extracts, neem and garlic were the most effective against *Bipolaris oryzae* at 1:1 dilution. All other plant extracts were also effective against *Bipolaris oryzae* at higher concentrations.

Key words: Plant extract, *Bipolaris oryzae, in vitro*, rice, neem, garlic

Introduction

In Bangladesh, rice is the staple food for the people. 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, but the average yield of rice in Bangladesh is only 1.87 tons/ha (BBS, 1999). So the average per hectare production of rice in Bangladesh is extremely low as compared to other rice growing countries of the world.

There are many constraints responsible for low yield of rice in Bangladesh. Brown spot is one of the 31 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 (Christen and Kaufmann, 1965). It also causes disease to the emerged rice seedlings (Mia and Mathur, 1983).

Various methods have been practised to control this pathogen. Use of plant extracts against plant disease is however, a recent approach to plant disease control. It helps to avoid environmental pollution by chemicals. In Bangladesh, a few attempts have been made to evaluate plant extracts against plant diseases (Hossain *et al*., 1993). Plant extracts reduced the seed infection (Suratuzzaman *et al*., 1994) by *Bipolaris oryzae* and produced seedling with longer shoots and roots (Alice and Rao, 1986). Plant extracts can reduce the growth of *Bipolaris oryzae* (Qamar and Chaudhary, 1991). Plant extracts exhibited fungitoxic properties and significantly reduced mycelial growth of *Helminthosporium oryzae* (Tripathi *et al*., 1983). In view of the above facts, the present study was undertaken in laboratory conditions to determine comparative efficacy of different plant extracts 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 paper 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 17 treatments (including control) following 3 (three) replications. Four selected plant extracts (treatments) were evaluated against *Bipolaris oryzae* are given below:

Common name	Scientific names	Plant parts used
Biskatali	*Polygonus hydropiper*	Leaf
Onion	*Allium cepa*	Bulb
Garlic	*Allium sativum*	Clove
Neem	*Azadirachta indica*	Leaf

The extracts were prepared by crushing the plant parts in a blender with distilled water in 1:1, 1:2, 1:4, 1:8 ratios (e.g. 1:1 = 100gm plant material crushed in 100 ml. water) (Hossain *et al*., 1997). The extracts were filtered by cheesecloth and centrifuged at 3500 rpm for 20 min. The supernatant was filtered through Whatman's No.1 filter paper (Bisht and Khulbe, 1995). The extracts thus obtained were kept in a refrigerator for subsequent uses.

Highest *Bipolaris oryzae* infected seed samples were used for seed treatment. At first, the seeds were soaked in the extracts of different dilutions separately. The extracts were then drained off and the seeds were placed on moist filter paper in petridishes and incubated at 22± 1°C under 12 hr cycle of alternate near ultra violet light and darkness. After ten days incubation the seeds were then observed under Stereo-microscope for the presence of *Bipolaris oryzae*. For each treatment, 200 seeds were tested.

Seedling vigour test by paper roll method were done. In this method the selected seed samples were treated with selected plant extracts of different dilution (1:1, 1:2, 1:4 and 1:8). 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 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 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 the formula of Geetha and Sivaprakasam (1993) given below:

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 two methods.

Poison food method: PDA was used for this experiment. The conical flasks and glass petridishes were sterilized in oven and the medium was then sterilized in autoclave. After autoclaving, the medium was taken out of the autoclave and allowed to cool to

50°C. Then the required amount of plant extracts were added into the media to get different concentrations of plant extracts. After mixing the plant extracts poisoned PDA was poured in sterilized petridish. For control plate, only water was used. Each treatment was replicated thrice. The diameter of the fungus was measured after 5 days of the incubation. The effect of plant extract on the growth of the fungus on agar medium was studied at 1:1, 1:2, 1:4 and 1:8 dilutions. 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 the 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 equidistant places by disc cutter. Four (1:1, 1:2, 1:4 and 1:8) dilutions were used in this method. Three drops of each plant extracts 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. Each treatment was replicated thrice. For control treatment, only water was used instead of plant extracts. The plates were then placed at 26± 2°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 the difference in controlling efficacy of plant extracts on *Bipolaris oryzae*.

Results and Discussion

Incidence of *Bipolaris oryzae* in rice seed collected from different villages of Mymensingh district is summarized in Table 1. Out of 12 seed samples tested all were infected with *Bipolaris oryzae*. 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%.

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

Samples	% Germination	% Seed-borne infection of *Bipolaris oryzae*
Bhabokhali	79.0ab	3.0
Bhavkhali	68.5c	5.5
Boira	80.5ab	2.5
Dhigerkanda	74.5abc	3.0
Barobillar par	70.00abc	4.5
Doribhavkhali 1	76.00abc	2.5
Raghobpur	72.00bc	3.0
Narianpur	76.5abc	2.5
Shutiakhali	80.0ab	3.0
Beltoli	78.0ab	3.5
Vijoynagor	79.0ab	3.0
Mahozompu	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

Results of seed treatment with plant extracts such as garlic, onion, neem and biskatali at different dilutions (1:1, 1:2, 1:4 and 1:8) are given in Table 2. The plant extracts increased seed germination. The highest germination was 72.5 percent when the seeds were treated with garlic extract at 1:1 dilution and followed by neem extract at 1:1 dilution. The lowest germination (67.0%) was observed, when the seeds were treated with Biskatali extract at 1:8 dilution which was same as control. Garlic extract and neem extract were effective against the seed-borne infection of *Bipolaris oryzae*. *Bipolaris oryzae* was best controlled with garlic extract where 91.7 percent fungal reduction was found at 1:1 dilution. Neem extract controlled 83.3 percent infection at 1:1 dilution. The lowest reduction (25.0 percent) was observed with biskatali extract at 1:8 dilution. Garlic extract and neem extract were almost equally effective in reducing seed-borne inocula of *Bipolaris oryzae*.

Effect of treating seeds with plant extracts on germination, root length, shoot length and vigour indices by paper roll method are presented in Table 3. The treatments were found to differ significantly. The highest root length (8.34 cm) was found in case of seeds treated with Garlic extract at 1:1 dilution followed by onion extract (8.25 cm) at 1:1 dilution. The lowest root length (6.74 cm) was observed when the seeds were treated with onion extract at 1:8 dilution. The highest shoot length (4.59 cm) was recorded when the seeds were treated with garlic extract at 1:1 dilution. The lowest shoot length (3.60 cm) was recorded with biskatali extract at 1:8 dilution. The vigour index (based on root length) was highest (679.9) when the seeds were treated with garlic extract at 1:1 dilution followed by neem extract at the same dilution, whereas the lowest count (476.2) was recorded with the biskatali extract at 1:8 dilution. The vigour index (based on shoot length) was highest (374.1) when the seeds were treated with garlic extract at 1:1 dilution followed by neem extract (361.3) at the same dilution whereas the lowest count (251.5) was recorded under the neem extract 1:8 dilution.

The results of Mycelial growth inhibition test by poison food method of plant extract on the growth of *Bipolaris oryzae* are presented in Table 4. The plant extracts and their different dilutions significantly inhibited the growth of *Bipolaris oryzae*. The highest inhibition was 43.32 percent found with neem extract at 1:1 dilution which was followed by garlic extract (36.36 percent) at 1:1 dilution. The lowest inhibition was 2.67 percent with biskatali and onion extract at 1:8 dilution.

The results of inhibition of the mycelial growth by cup method are given in Table 5. All plant extracts and their different dilutions significantly inhibited the mycelial growth of *Bipolaris oryzae*. The highest inhibition was 41.01 percent with neem extract at 1:1 dilution and followed by garlic extract which inhibited 38.76 percent at the same dilution. The lowest inhibition was 3.65 percent with biskatali at 1:8 dilution.

The efficacy tests of plant extracts were done by seed treatment and mycelial growth inhibition test under laboratory conditions against *Bipolaris oryzae* in rice. The testing procedures also were carried out in accordance with ISTA rules. 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 percent 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 percent *Helmithosporium oryzae* infection was recorded in the collected samples from BAU farm during 1970.

Efficacy of plant extracts for controlling seed-borne *Bipolaris oryzae*, garlic extract and neem extract was found effective. Excellent results were also obtained when the seeds were treated with garlic extract at 1:1 dilution. Neem extract (1:1 dilution) ranked next in controlling the seed-borne *Bipolaris oryzae*. Garlic (1:1 dilution) and neem extract (1:1 dilution inhibited the growth of the pathogen by 91.7 percent and 83.3 percent over control respectively. The present findings are in agreement with the findings of Suratuzzaman *et al.* (1994), who obtained good effect of garlic extract for controlling *Drechslera oryzae*. Miah *et al.* (1990) reported that *Drechslera oryzae* was best controlled by *Allium sativum* and *Azadirachta indica* also controlled this pathogen significantly over control.

Garlic and neem extracts were more effective at 1:1 dilution for seed germination and seedling vigour. Alice and Rao (1986) obtained identical results with garlic extract. Garlic extract treated seeds produced seedlings with longer shoots and roots.

Efficacy of plant extracts were also evaluated by poison food method and cup method. In poison food method, all the plant extracts showed inhibitory effect against *Bipolaris oryzae*. The

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

Treatments	Dilution	% Germination	Total fungal population (%)	Fungal reduction (%)
Control	0	67.0	6.0a	-
Biskatali	1:1	70.0	2.0bcd	66.7
	1:2	69.5	2.5bcd	58.3
	1:4	67.5	3.5a-d	41.7
	1:8	67.0	4.5ab	25.0
Onion	1:1	71.5	1.5bcd	75.0
	1:2	68.0	3.0a-d	50.0
	1:4	67.5	4.5ab	25.0
	1:8	67.5	4.0abc	33.3
Garlic	1:1	72.5	0.5d	91.7
	1:2	71.0	1.0cd	83.3
	1:4	69.5	2.0bcd	66.7
	1:8	69.5	3.0bcd	50.0
Neem	1:1	72.0	1.0cd	83.3
	1:2	70.0	1.5bcd	75.0
	1:4	69.0	2.5bcd	58.3
	1:8	67.5	3.5a-d	41.7
LSD (p = 0.05)		NS	0.631	NS

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

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

Treatments	Dilution	% Germination	Root length (cm)	Shoot length (cm)	Vigour index based on root length and percent germination	Vigour index based on shoot length and percent germination
Control	0.0	69.0	6.78 ef	3.60 fg	467.5 f	230.6j
Biskatali	1:1	75.5	7.23b-e	3.89def	545.6cd	293.9e-h
	1:2	74.0	7.14b-f	3.71efg	528.0cde	274.1ghi
	1:4	72.5	6.88def	3.69efg	498.4def	267.1ghi
	1:8	70.0	6.80def	3.60fg	476.2ef	252.3ij
Onion	1:1	76.5	8.25a	4.44ab	631.3ab	336.1bcd
	1:2	75.5	7.55b	4.24a-d	569.6c	319.8cde
	1:4	73.5	7.09-f	3.88def	520.7c-f	284.6f-i
	1:8	71.5	6.74f	3.72efg	481.5ef	266.0hij
Garlic	1:1	81.5	8.34a	4.59a	679.9a	374.1a
	1:2	80.0	7.98a	4.34abc	638.8cab	347.3abc
	1:4	75.5	7.34bc	4.15bcd	554.4c	313.4c-f
	1:8	74.0	6.72f	3.92def	497.4def	290.5e-h
Neem	1:1	81.0	8.09a	4.46ab	655.5ab	361.3ab
	1:2	78.5	8.00a	4.22a-d	628.2b	331.3bcd
	1:4	74.5	7.25bcd	4.05cde	540.2cd	302.0d-g
	1:8	73.0	6.89c-f	3.69efg	502.9def	251.5ij
LSD (p= 0.01)		NS	0.408	0.35	46.30	31.59

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

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

Treatments	Dilutions	Mean inhibition of growth (cm)	Mean percent inhibition of growth
Control	0	3.74 a	-
Biskatali	1:1	2.90ef	22.46
	1:2	3.13cd	16.31
	1:4	3.31b	11.50
	1:8	3.64a	2.67
Onion	1:1	2.50gh	32.35
	1:2	2.77f	25.94
	1:4	3.01de	19.52
	1:8	3.64a	2.67
Garlic	1:1	2.38i	36.36
	1:2	2.59g	30.75
	1:4	3.07d	17.91
	1:8	3.26bc	12.83
Neem	1:1	2.12j	43.32
	1:2	2.15j	42.51
	1:4	2.44hi	34.58
	1:8	3.03de	18.98
LSD (p= 0.01)		0.14	

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

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

Treatments	Dilutions	Mean inhibition of growth (cm)	Mean percent inhibition of growth
Control	0	3.56 a	-
Biskatali	1:1	2.54fg	28.65
	1:2	3.01cde	15.45
	1:4	3.26a-d	8.43
	1:8	3.43a	3.65
Onion	1:1	2.25ghi	36.67
	1:2	2.59fg	27.25
	1:4	3.06b-e	14.04
	1:8	3.34abc	6.18
Garlic	1:1	2.18hi	38.76
	1:2	2.34ghi	34.26
	1:4	2.51fgh	29.49
	1:8	3.35ab	5.90
Neem	1:1	2.10i	41.01
	1:2	2.39ghi	32.86
	1:4	2.73ef	23.31
	1:8	2.95de	17.13
LSD (p= 0.01)		0.313	

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

best inhibitory effect was observed in neem extract at 1:1 dilution where 43.32 percent growth inhibition of the fungus was recorded.

In cup method, all the plant extracts give inhibitory effect against *Bipolaris oryzae*. The best inhibitory effect was observed in neem extract at 1:1 dilution. The other plant extracts also showed their best efficacy at 1:1 dilution. This agreed partially with the findings of Bisht and Khulbe (1995) where garlic gave the best results among plant extracts used. Ganguly (1994) obtained good inhibitory effect of *Azadirachta indica* against *Helmithosporium oryzae*.

From the above study, it has been clearly observed that all plant extracts were effective against *Bipolaris oryzae* at higher concentration. Among them neem and garlic were the most effective at 1:1 dilution. So, the garlic and the neem extracts may be used for controlling *B. oryzae* of rice.

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