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Isolation, characterization and management of brown spot disease of rice

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Abstract
Rice brown spot is the one of the most important disease of rice, a major cause of Bengal famine. The brown spot infected leaf samples were collected from five major rice growing location of Tamil Nadu. Based on the colony character, the pathogen was isolated and characterized into 3 groups; greyish with cottony mycelium mixture of grey and white mycelium with fluffy growth and greyish cottony mycelium with white spots. The condium was slightly curved, multi-septate and fusiform in shape. PCR based method was used for molecular characterization of the brown spot pathogen using universal primers ITS-1/4 and the partial sequences were obtained and submitted in NCBI database. *In vitro* and *in vivo* evaluation of twelve fungicides were carried, among the twelve fungicides, Hexaconazole 5% EC and Tebuconazole 25% + Trifloxystrobin 50% WP and Zineb 68% + Hexaconazole 25% WG were effective even at 50 ppm concentration with 100 per cent inhibition over control.

Keywords: Brown spot, fungicides, management, rice

Introduction
Rice is the most important cereal crop next to the wheat in area and production which is the primary source of food for nearly 90% of world human population, especially in Asia. Globally, India is the second largest producer of rice in area and production next to China. Rice is an important grain, which is enriched with high amount of carbohydrate, protein and fats. It provides more than one – fifth of calories consumed by the human's worldwide (Jatoi *et al.,* 2018) [4]. There is continues increase in the global demand for rice grain due to continues increase in the world population. The global demand is expected to be 852 million tons by the year 2035 with the current production approximately 770 million tons. In order to fulfill the demand, there is need to intensify the production technology. But with introduction of improved technologies and high yielding varieties, the crop become susceptible to many biotic and abiotic stresses, especially biotic stresses like diseases (Sunder *et al.,* 2014) [12]. Among several diseases of rice, brown spot of rice caused by *Bipolaris oryzae* (Breda de Haan) Shoemaker, (Telemorph – *Cochliobolus miyabeanus*) was major problem eventually caused sustainable losses both in quality and quantity (Hossain *et al*., 2011) [3]. Besides, the disease instigated the Bengal famine in 1943 which took away the lives of 2 million people around Bengal region before partition. The disease could extent the severity to the crop up to 90% where it also reduces the growth, grain discoloration and reduces the market quality of the rice grain (Valarmathi and Ladhalakshmi, 2018) [13]. As far as the management for brown spot of rice is concerned, strategies were limitedly explored where resistance cultivars were not as much developed. However, the chemical management of *B. oryzae* was very quick, effective, low cost control measure (Kumar *et al*., 2017) [6]. Earlier recommended chemicals like Zineb, was not effective under high inoculums pressure. In last decades, a large number chemical with different mode of action and target in combination were applied to reduce the disease severity and for effective management of the brown spot disease (Hossain *et al*., 2011) [3]. Hence, the present study is focused on collection, morphological and molecular characterization of the brown spot pathogen and effective management of the brown spot of rice.

Materials and Methods
Collection of brown spot infected leaf samples
The rice brown spot infected leaf samples were collected from the major rice growing location of Tamil Nadu *viz.,* Paddy breeding station (Coimbatore), Tamil Nadu Rice Research Institute (Tanjavur), Hybrid Rice Evaluation Centre (Gudalur), Agricultural Research Station (Bavanisagar), and Farmer's field (Dharapuram) form the varieties CO 43, ADT 39, CO 51, Bhavani and CO 50 respectively.

Isolation of *B. oryzae* from infected leaf samples

The fungus was isolated from the brown spot infected leaf sample. The infected leaf samples were washed with sterile water to remove the dirt on the surface of the leaves and infection portion of the leaf along with green portion was cut into small pieces. These were dipped in 1% sodium hypochlorite for 1 minute and followed by subsequent sterile distilled water wash for three times to remove the excess sodium hypochlorite. The leaf bits were air dried on the blotter paper to remove the excess water the leaf surface. Then the leaf bits were transferred into the sterile petri-plate containing Potato Dextrose Agar medium amended with streptomycin sulphate to avoid the bacterial contamination under aseptic condition. The petri-plates were incubated at 28±2°C for 3 days and the actively growing mycelium was sub-cultured. The isolated fungi were purified by single spore method and purified cultures were maintained in PDA slants at 4°C for further use. Five isolates were designated as BO-1 for Coimbatore, BO-2 for Tanjavur, BO-3 for Gudalur, BO-4 for Bavanisagar and BO-5 for Dharapuram.

Morphological characterization of *B. oryzae*

Morphological characterizations of five isolates were carried out in PDA by incubated at 28±2°C for 7 days. Characters like colony growth, mycelial colour, sporulation, conidium size, shape, colour and conidiophore characters were observed. All five isolates were grown in PDA medium for 5-7 days. A 9mm mycelial disc from 7 days old culture was placed at centre of sterilized glass slide under aseptic condition on moist sterile Petri-plate and incubated at 25±2°C for 3 days with alternate 12 hrs light and dark period. After 3 days of incubation, the spore suspension was collected using sterile distilled water and examined under compound microscope (Kumari *et al.,* 2015) [7].

Pathogenicity test

The seed materials were collected form Paddy Breeding Station, Coimbatore. CO-50 variety was moderately susceptible to brown spot disease, where used for pathogenicity test. The plants were grown glass house condition at 28°C with relative humidity of 80%. BO-1 isolate was taken for testing pathogenicity and the pathogen was mass multiplied in sterilized paddy chaff grain in a 250 ml conical flask for 15 days. The spore suspension was collected from the conical flask using sterile water by vigorous shaking and filtered through muslin cloth. The spore concentration was adjusted to 5×10^4 using haemocytometer. Thirty day old plants were sprayed with conidial suspension along with two drops tween-20 and control plant was sprayed with sterile distilled water with tween-20. The sprayed plants were covered with polythene sheets to maintain adequate humidity and temperature. Plants were observed for symptom expression (Nazari *et al.,* 2015) [9].

Molecular characterization of *B. oryzae*

DNA extraction

The brown spot pathogen isolates were grown in Potato Dextrose Broth for 15 days and the mycelial mats were harvested through filter paper. The mycelial mats were dried at room temperature for 24 hours. The DNA was extracted by CTAB method as per Saghai-Maroof *et al.* (1984) [10]. The extracted genomic DNA electrophoresed on 0.8% agarose gel for 30 minutes along with loading dye and presence of genomic DNA was documented on image analyzer.

PCR analysis

Universal primers ITS-1(5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'- TCCTCCGCTTATTGATATGC-3') were used for screening all five isolates of *B. oryzae*. The PCR amplification reaction was carried out for final volume of 30µl, which consists of 15 µl Master mix, 3 µl of forward primes ITS-1 and 3 µl of reverse primer ITS-4 and 6 µl of sterile distilled water. The PCR amplification was carried out in thermo cycler with following condition; initial denaturation at 94°C for 4 minutes, and followed by 40 cycles of denaturation (94°C for 1 minute), annealing (53°C for 45 seconds) and extension (72°C for 1 minutes) and final extension at 72°C for 10 minutes, then hold at 4°C. Then the PCR products were electrophoresed in 1.2% agarose gel for 1 hour in 1× TAE buffer stained with ethidium bromide. The gel was visualized using UV transilluminator in gel documenting unit.

***In vitro* evaluation of fungicides against *B. oryzae* in a solid medium**

For testing the efficacy of twelve fungicides against brown spot pathogen *viz.,* Carbendazim 50% WP, Propineb 70% WP, Hexaconazole 5% EC, Tebuconazole 25% WG, Tricyclazole 75% WP, Propiconazole 25% EC, Kresoxim-methyl 44.3% SC, Isoprothiolan 49% EC, Difenconazole 25% EC, Zineb 68% + Hexaconazole 4% WP, Tebuconazole 50% + Trifloxystrobin 25% WG and Carbendazim 25% + Mancozeb 50% WP were used at different concentration of 50, 100, 250, 500, 1000, 1500 ppm by Poison Food Technique (Dhingra and Sinclair, 1995) [1]. A 9 mm mycelial disc was placed at the centre of the Petri-plate containing PDA medium amended with fungicides and control plate was maintained without fungicides. Three replications were maintained for each treatment. The plates were maintained at 28±2°C for 7 days. The mycelial growth was recorded after 7 day of incubation. The efficacy was expressed as per cent inhibition over control using the formula.

$$\text{Per cent Inhibition} = \frac{C - T}{C} \times 100$$

C – Mycelial growth in control, T – Mycelial growth in treatment

***In vitro* evaluation of fungicides against *B. oryzae* in a liquid medium**

The efficacy of the fungicides was tested in liquid Potato Dextrose Broth (PDB) medium with different concentration *viz.,* 50, 100, 250, 500, 1000 and 1500 ppm. Carbendazim 50% WP, Propineb 70% WP, Hexaconazole 5% EC, Tebuconazole 25% WG, Tricyclazole 75% WP, Propiconazole 25% EC, Kresoxim-methyl 44.3% SC, Isoprothiolan 49% EC, Difenconazole 25% EC, Zineb 68% + Hexaconazole 4% WP, Tebuconazole 50% + Trifloxystrobin 25% WG and Carbendazim 25% + Mancozeb 50% WP were used for testing efficacy in liquid medium. A 9 mm mycelial disc from the 7 days old culture was transferred into the 250 ml conical flask containing PDB amended with the fungicides at above mentioned concentration. The conical flasks were maintained at 28±2°C for 15 days. After 15 days of incubation, the wet and dry mycelial weights were recorded.

Evaluation of fungicides against brown spot of rice under field condition

The field trial was conducted at experimental plot of Paddy breeding station (PBS), Coimbatore during the year 2018-2019. Rice variety BPT-5204 was used for the study and the seed samples were collected from PBS, Coimbatore. The field layout was made in Randomized Block Design (RBD) with three replication having plot size of 5 × 2 m^2 with row to row and plant to plant spacing of 15 × 15 cm. The NPK application and other practices were done as per recommendation in crop protection guide. The following ten treatments *viz.,* T_1 = Hexaconazole 5% EC @ 0.125 ml/litre, T_2 = Tebuconazole 25% WG @ 1.5ml/litre, T_3 = Tricyclazole 75% WP @ 0.8g/litre, T_4 = Propiconazole 25% EC @ 0.3ml/litre, T_5 = Difenconazole 25% EC @ 0.5ml/litre, T_6 = Zineb 68% + Hexaconazole 25% WG @ 0.625g/litre, T_7 = Tebuconazole50% + Trifloxystrobin 25% WG @ 0.04g/litre, T_8 = Carbendazim 25% + Mancozeb 50% WP @ 2.5ml/litre, T_9 = Carbendazim 50% WP @ 2.0g/litre and T_{10} = Control were compared against brown spot under artificial inoculation. The treatments concentration was fixed based on the *in vitro* screening result and recommended dosage for the rice diseases. The spraying was done on initial stage of disease appearance after 3 days of pathogen inoculation in early morning. Observation on disease severity and Per cent Disease Index were made at 7 days interval. The Per cent Disease Index (PDI) was calculated using the following formula.

$$Percent\ Disease\ Index = \frac{\text{Summation of all numerical rating}}{Total\ number\ of\ plants\ observed \times Maximum\ rating} \times 100$$

The Standard Evaluation Scale for brown spot used was 0-9 (IRRI, 2013) where, 0 = No incidence, 1 = Less than 1% affected leaf area, 2 = 1-3% affected leaf area, 3 = 4-5% affected leaf area, 4 = 6-10% affected leaf area, 5 = 11-15% affected leaf area, 6 = 16-25% affected leaf area, 7 = 25-50 affected leaf area, 8 = 51-75% affected leaf area, 9 = 75-100% affected leaf area. The Disease rating was taken from 25 leaves of randomly tagged five plants in each plot. The per cent increase in the yield in treated and control were calculated using the formula.

$$Percent\ Increase\ in\ yield = \frac{T - C}{C} \times 100$$

T – Estimated yield in the fungicides treated plot,
C – Estimated yield in untreated plot.

Result

Morphological characterization of *B. oryzae*

Based on the colony morphology and growth on the PDA medium, all five isolates were divided into 3 categories: Isolate BO-1 was having greyish with cottony mycelium where as isolates BO-2, 4, 5 were having mixture of grey and white mycelium with fluffy growth and Isolate BO-3 was having greyish cottony mycelium with whit spots (Fig.1). Based on the mycelial growth habitat, isolates BO-1, 2, 4 were able to cover full plate of 9 cm within seven days of incubation and regarded as fast growing isolates and isolates BO-3, 5 were comparatively slow grown than other isolates which takes nearly 10 days for completely covering 9 mm Petri plates (Table.1). All five isolates produced spore on the Petri-plate on PDA medium. The fungus appeared initially as white mycelium, later turn dark brown with septation and conidiophore arises singly or group, multi-septate, brown in colour which bears conidia on the tips (Fig.2). Conidia were curved or slightly curved; initially hyaline and later on maturity turns brown in colour, fusiform with hilum at base (Fig. 3). Conidia size varies from 95- 80µm to 15-10 µm and germinated at both end of the conidium (Fig. 4).

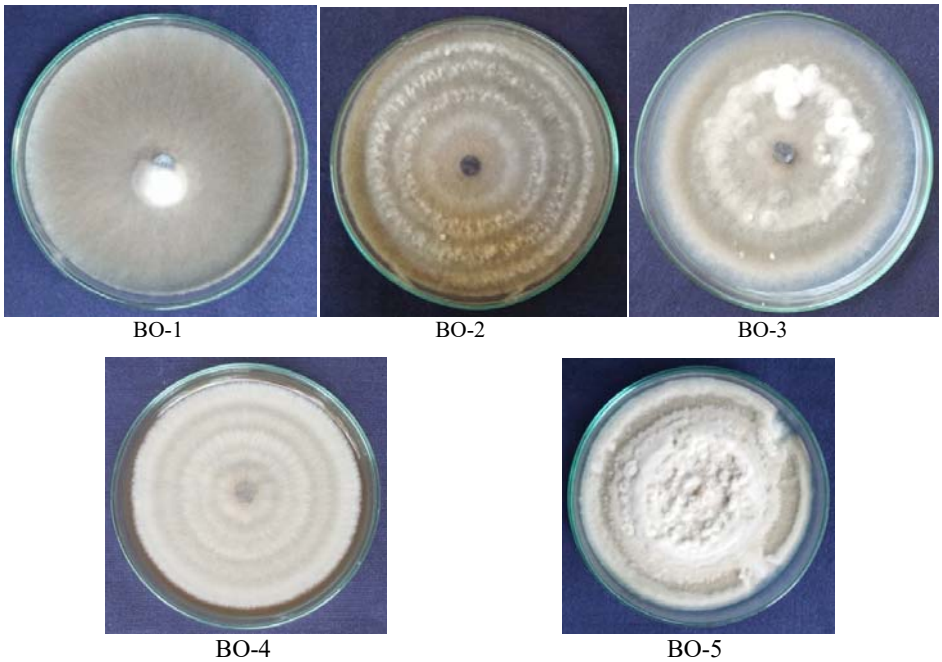

Fig 1: Variation in colony morphology of brown spot isolates.

Fig 2: Conidial arrangement on the conidiophore

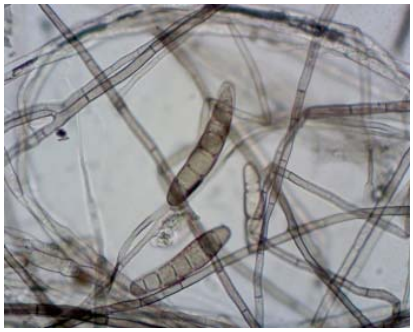

Fig 3: Conidial character of *B. oryzae*

Fig 4: Bipolar germination of conidia

Pathogenicity test

Rice variety CO-50, moderately susceptible to brown spot was used for the pathogenicity test. The symptom started to appear after one day of inoculation. The pathogen initially produced pin head size spot, which gradually enlarged to form oval shaped spot after 3 days of inoculation (Fig. 5). On later dates, the spots coalesces together resulting in drying up of leaves.

Fig 5: Pinhead size brown spot in the artificially inoculated leaves

Molecular characterization of *B. oryzae*

The DNA was isolated from the five isolated by CTAB method and checked in 1.2% agarose gel. The ITS1 and ITS4 primers amplified a fragment of 570 bp corresponding to the ITS1 and ITS4 regions of the rDNA for the *B. oryzae*. Five isolates were examined for the amplification of ITS region and all these isolates showed amplified product with size range of 570 bp. The partial sequenced of rDNA were obtained and it were submitted in the National Centre for Biotechnology Information (NCBI), Gene Bank, New York, USA and accession numbers were obtained.

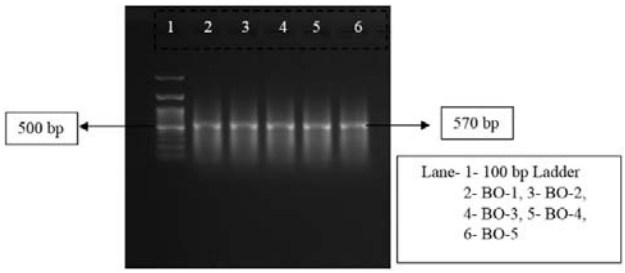

Fig 6: PCR amplification of ITS region using universal primers (ITS-1/4)

***In vitro* evaluation of fungicides against *B. oryzae* in a solid medium**

Twelve fungicides were tested against *B. oryzae* at six different concentration of 50, 100, 250, 500, 1000 and 1500 ppm. The data showed significance ($P < 0.05$) for all twelve fungicides tested under *in vitro* condition. Among the twelve fungicides, Hexaconazole 5% EC and Tebuconazole 25% + Trifloxystrobin 50% WG showed complete inhibition in all six concentration Where as Hexaconazole 4% + Zineb 68% WP and Propiconazole 25% EC showed 100% inhibition at 250 and 500 ppm respectively. Carbendazim 50% WP, Tricyclazole 75% WP, Tebuconazole 25% WG, Kresoxim-methyl 44.3% SC, Isoprothiolan 49% EC and Difenconazole 25% EC showed partial inhibition of 84.44, 85.56, 85.56, 72.22, 69.63 and 85.56% at 1500 ppm respectively when compared to control and Carbendazim 25% + Mancozeb 50% WP and Propineb 70% WP were less effective than all other fungicides tested. Among the twelve fungicides, Hexaconazole 5% EC and Tebuconazole 25% + Trifloxystrobin 50% WG were the most effective fungicides against brown spot of rice (Table. 2 & 3).

***In vitro* evaluation of fungicides against *B. oryzae* in a liquid medium**

In PDB, the efficacy of the twelve fungicides was tested against brown spot at six different concentrations. Among all fungicides, Hexaconazole 5% EC, Propiconazole 25% EC, Isoprothiolan 49% EC, Difenconazole 25% EC, Zineb 68% + Hexaconazole 4% WP, and Tebuconazole 50% + Trifloxystrobin 25% WG were effectives in all concentration with 100% inhibition over control, whereas Carbendazim 50% WP, Tricyclazole 75% WP, Tebuconazole 25% WG, and

Carbendazim 25% + Mancozeb 50% WP showed complete inhibition at 500 ppm and Isoprothiolan 49% EC showed complete inhibition from 1000 ppm onwards. The fungicides, Propineb 70% WP and Kresoxim-methyl 44.3% SC were showing partial inhibition over control in all concentration (Table. 4 & 5).

Evaluation of fungicides against brown spot of rice under field condition

The result for field evaluation of fungicides against brown spot was shown in the Table 6. The disease severity, percent disease incidence, percent disease reduction over control and yield were recorded. Among the result shown, maximum disease reduction over control (71.11%) and yield were exhibited in T_1- Hexaconazole 5% EC @ 0.125ml/litre (14.67%) followed by T_7 = Tebuconazole 50% + Trifloxystrobin 25% WG @ 0.04g/litre (22.67%) and T_6 = Zineb 68% + Hexaconazole 25% WG @ 0.625g/litre (28.44%). The minimum disease reduction over control, higher percent disease incidence and disease severity were exhibited in T_3 = Tricyclazole 75% WP @ 0.8g/litre (59.11%) followed by T_8 = Carbendazim 25% + Mancozeb 50% WP @ 2.5ml/litre (58.67%) and T_9 = Carbendazim 50% WP @ 2.0g/1 litre (51.56%). In this experiment, all fungicides showed controlling of disease. Among the fungicides tested, Hexaconazole 5% EC alone without combination was effective one in controlling the brown spot disease (Table.6).

Table 1: Colony morphology and mycelial growth of *B. oryzae*

S. No	Isolates	Radial mycelial growth (cm)*				Colony character	Growth habitat	sporulation
		1st day	3st day	5st day	7st day			
1.	BO-1	2.0^{a} (1.41)	5.5^{a} (2.35)	7.9^{a} (2.81)	9.0^{a} (3.00)	Grayish and cottony mycelium	Fast	+
2.	BO-2	1.8^{b} (1.34)	5.0^{b} (2.24)	7.7^{a} (2.77)	9.0^{a} (3.00)	mixture of grey and white mycelium with fluffy growth	Fast	+
3.	BO-3	1.4^{c} (1.18)	4.5^{c} (2.12)	6.1^{b} (2.47)	8.0^{b} (2.83)	Grayish, cottony mycelium with white dots	Moderate	+
4.	BO-4	1.9^{b} (1.38)	5.4^{a} (2.32)	7.7^{a} (2.77)	9.0^{a} (3.00)	mixture of grey and white mycelium with fluffy growth	Fast	+
5.	B0-5	1.3^{d} (1.14)	4.3^{d} (2.07)	5.9^{c} (2.43)	7.2^{c} (2.68)	mixture of grey and white mycelium with fluffy growth	Moderate	+
	SEd	0.07	0.08	0.08	0.09			
	CD (0.05)	0.15	0.18	0.17	0.19			

* Mean of the three replication

Figures in the parentheses represent square root transformation.

Means in the column followed by same superscript letters are not significantly different according to DMRT

Table 2: *In vitro* evaluation of fungicides against mycelial growth of brown spot pathogen

S. No	Treatments	Mycelial growth after 7 days of incubation in diameter (cm)*					
		50 ppm	100 ppm	250 ppm	500 ppm	1000 ppm	1500 ppm
1.	Carbendazim 50% WP	5.57^{bc}	4.20^{bc}	3.13^{bc}	2.50^{bc}	2.00^{bc}	1.40^{bc}
2.	Propineb 70% WP	6.07^{c}	5.70^{c}	4.20^{c}	3.07^{c}	2.43^{c}	1.77^{c}
3.	Hexaconazole 5% EC	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
4.	Tebuconazole 25% WG	3.33^{b}	2.83^{b}	2.43^{b}	2.10^{b}	1.80^{b}	1.30^{b}
5.	Tricyclazole 75% WP	4.83^{bc}	3.80^{bc}	3.20^{bc}	2.87^{bc}	2.33^{bc}	1.33^{bc}
6.	Propiconazole 25% EC	2.17^{a}	1.83^{a}	1.10^{a}	0.00^{a}	0.00^{a}	0.00^{a}
7.	Kresoxim-methyl 44.3% SC	4.43^{bc}	4.00^{bc}	3.63^{bc}	3.23^{bc}	2.87^{bc}	2.50^{bc}
8.	Isoprothiolan 49% EC	4.43^{bc}	3.93^{bc}	3.77^{bc}	3.63^{bc}	3.17^{bc}	2.73^{bc}
9.	Difenconazole 25% EC	4.87^{bc}	3.93^{bc}	3.07^{bc}	2.30^{bc}	1.87^{bc}	1.27^{bc}
10.	Zineb 68% + Hexaconazole 4% WP	1.40^{a}	1.13^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
11.	Tebuconazole 50% + Trifloxystrobin 25% WG	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}	0.00^{a}
12.	Carbendazim 25% + Mancozeb 50% WP	7.93^{c}	5.80^{c}	4.17^{c}	3.50^{c}	2.10^{c}	1.40^{c}
13.	Control	9.00^{d}	9.00^{d}	9.00^{d}	9.00^{d}	9.00^{d}	9.00^{d}
	SEd	0.47	0.36	0.31	0.10	0.07	0.06
	CD (0.05)	0.96	0.73	0.63	0.21	0.14	0.12

* Mean of the three replication

Means in the column followed by same superscript letters are not significantly different according to DMRT

Table 3: *In vitro* evaluation of fungicide against brown spot pathogen

S. No	Treatments	Percent inhibition after 7 days of incubation in diameter (%)*					
		50 ppm	100 ppm	250 ppm	500 ppm	1000 ppm	1500 ppm
1.	Carbendazim 50% WP	38.15^{bc}	53.33^{bc}	65.19^{bc}	72.22^{bc}	77.78^{bc}	84.44^{bc}
2.	Propineb 70% WP	32.59^{c}	36.67^{c}	53.33^{c}	65.9^{bc}	72.96^{c}	80.37^{c}
3.	Hexaconazole 5% EC	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}
4.	Tebuconazole 25% WG	62.96^{b}	68.52^{b}	72.96^{b}	76.67^{b}	80.00^{b}	85.56^{b}
5.	Tricyclazole 75% WP	46.30^{bc}	57.78^{bc}	64.44^{bc}	68.15^{bc}	75.19^{bc}	85.19^{bc}
6.	Propiconazole 25% EC	75.93^{a}	79.63^{a}	87.78^{a}	100.00^{a}	100.00^{a}	100.00^{a}
7.	Kresoxim-methyl 44.3% SC	50.74^{bc}	55.56^{bc}	59.63^{bc}	64.07^{bc}	68.15^{bc}	72.22^{bc}
8.	Isoprothiolan 49% EC	50.74^{bc}	56.30^{bc}	58.15^{bc}	59.63^{bc}	64.81^{bc}	69.63^{bc}
9.	Difenconazole 25% EC	45.93^{bc}	56.30^{bc}	65.93^{bc}	74.44^{bc}	79.26^{bc}	85.56^{bc}

10.	Zineb 68% + Hexaconazole 4% WP	79.26[a]	87.78[a]	100.00[a]	100.00[a]	100.00[a]	100.00[a]
11.	Tebuconazole 50% + Trifloxystrobin 25% WG	100.00[a]	100.00[a]	100.00[a]	100.00[a]	100.00[a]	100.00[a]
12.	Carbendazim 25% + Mancozeb 50% WP	11.85[c]	35.56[c]	53.70[c]	61.11[c]	76.67[c]	84.44[c]
13.	Control	0.00[d]	0.00[d]	0.00[d]	0.00[d]	0.00[d]	0.00[d]
	SEd	5.17	3.96	3.41	1.11	0.77	0.21
	CD (0.05)	10.65	8.15	7.02	2.28	1.59	0.42

* Mean of the three replication
Means in the column followed by same superscript letters are not significantly different according to DMRT

Table 4: *In vitro* evaluation of fungicides against *B. oryzae* in a liquid medium (Wet weight)

S. No	Treatments	Wet mycelial weight after 7 days of incubation in grams (g)*					
		50 ppm	100 ppm	250 ppm	500 ppm	1000 ppm	1500 ppm
1	Carbendazim 50% WP	3.451[ab]	0.207[ab]	0.00[ab]	0.00[ab]	0.00[ab]	0.00[ab]
2	Propineb 70% WP	4.091[b]	1.625[b]	0.893[b]	0.501[b]	0.308[b]	0.171[b]
3	Hexaconazole 5% EC	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]
4	Tebuconazole 25% WG	0.131[a]	0.073[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]
5	Tricyclazole 75% WP	0.872[ab]	0.451[ab]	0.224[ab]	0.00[ab]	0.00[ab]	0.00[ab]
6	Propiconazole 25% EC	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]
7	Kresoxim-methyl 44.3% SC	1.850[b]	1.659[b]	1.212[b]	1.011[b]	0.789[b]	0.509[b]
8	Isoprothiolan 49% EC	2.880[b]	2.274[b]	1.778[b]	0.422[b]	0.00[ab]	0.00[ab]
9	Difenconazole 25% EC	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]
10	Zineb 68% + Hexaconazole 4% WP	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]
11	Tebuconazole 50% + Trifloxystrobin 25% WG	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]
12	Carbendazim 25% + Mancozeb 50% WP	3.222[ab]	2.770[ab]	0.00[ab]	0.00[ab]	0.00[ab]	0.00[ab]
13	Control	4.671[c]	4.671[c]	4.671[c]	4.671[c]	4.671[c]	4.671[c]
	SEd	0.04	0.04	0.03	0.02	0.01	0.01
	CD (0.05)	0.09	0.08	0.05	0.04	0.03	0.02

* Mean of the three replication.
Means in the column followed by same superscript letters are not significantly different according to DMRT

Table 5: *In vitro* evaluation of fungicides against *B. oryzae* in a liquid medium (Dry weight)

S. No	Treatments	Dry mycelial weight after 7 days of incubation in grams (g)*					
		50 ppm	100 ppm	250 ppm	500 ppm	1000 ppm	1500 ppm
1	Carbendazim 50% WP	0.520[ab]	0.028[ab]	0.00[ab]	0.00[ab]	0.00[ab]	0.00[ab]
2	Propineb 70% WP	0.598[b]	0.301[b]	0.139[b]	0.041[b]	0.020[b]	0.003[b]
3	Hexaconazole 5% EC	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]
4	Tebuconazole 25% WG	0.010[a]	0.003[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]
5	Tricyclazole 75% WP	0.136[ab]	0.029[ab]	0.009[ab]	0.00[ab]	0.00[ab]	0.00[ab]
6	Propiconazole 25% EC	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]
7	Kersoxim-methyl 44.3% SC	0.302[b]	0.289[b]	0.219[b]	0.182[b]	0.099[b]	0.031[b]
8	Isoprothiolan 49% EC	0.389[b]	0.301[b]	0.221[b]	0.072[b]	0.00[ab]	0.00[ab]
9	Difenconazole 25% EC	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]
10	Zineb 68% + Hexaconazole 4% WP	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]
11	Tebuconazole 50% + Trifloxystrobin 25% WG	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]	0.00[a]
12	Carbendazim 25% + Mancozeb 50% WP	0.472[ab]	0.370[ab]	0.00[ab]	0.00[ab]	0.00[ab]	0.00[ab]
13	Control	0.658[c]	0.658[c]	0.658[c]	0.658[c]	0.658[c]	0.658[c]
	SEd	0.026	0.022	0.012	0.008	0.006	0.003
	CD (0.05)	0.053	0.045	0.024	0.017	0.012	0.005

* Mean of the three replication
Means in the column followed by same superscript letters are not significantly different according to DMRT

Table 6: Evaluation of fungicides against brown spot of rice under field condition

S. No	Fungicides	Dosage (ml or g/ litre of water)	Disease severity	PDI	Percent disease reduction over Control	Grain yield (kg/plot)	Percent increase in yield
1.	Hexaconazole 5% EC	0.125 ml	0.33[a]	14.67[a]	79.37[a]	6.488[a]	23.58[a]
2.	Tebuconazole 25% WG	1.5 ml	4.00[ef]	43.11[f]	38.37[f]	6.088[bc]	15.96[cd]
3.	Tricyclazole 75% WP	0.8 g	6.33[h]	59.11[i]	16.87[i]	5.333[e]	1.58[f]
4.	Propiconazole 25% EC	0.3 ml	2.67[cd]	38.67[d]	45.61[d]	6.166[bc]	17.45[cd]
5.	Difenconazole 25% EC	0.5 ml	3.33[de]	46.22[e]	35.01[e]	6.133[bc]	16.82[cd]
6.	Zineb 68% + Hexaconazole 4% WP	0.625g	2.00[bc]	28.44[c]	60.02[c]	6.266[ab]	19.35[bc]
7.	Tebuconazole 50% + Trifloxystrobin 25% WG	0.04g	1.00[ab]	22.67[b]	68.12[b]	6.433[a]	22.53[ab]
8.	Carbendazim 25% + Mancozeb 50% WP	2.5g	6.00[gh]	58.67[h]	17.49[h]	5.800[d]	10.48[e]
9.	Carbendazim 50% WP	2.0g	5.00[fg]	51.56[g]	27.42[g]	6.000[cd]	14.29[de]
10.	Untreated (control)		8.00[i]	71.11[j]	0.0[j]	5.250[e]	0.0[f]
	SEd		0.59	0.33	0.34	0.10	1.88
	CD(0.05)		1.24	0.68	0.70	0.21	3.92

Means in the column followed by same superscript letters are not significantly different according to DMRT

Discussion

In this study, the rice brown spot infected leaf samples were collected from different location of Tamil Nadu. The pathogen was isolated, characterized based on the morphological and cultural characters. Five isolates were categorized into 3 groups: greyish with cottony mycelium mixture of grey and white mycelium with fluffy growth and greyish cottony mycelium with white spots. The mycelium was brown, branched and septate which gives rise to conidiophore singly or group which bears conidium at tip. Conidia were multi-septate, brown, slightly curved and fusiform in shape. Kumar *et al.,* (2011) grouped the *B. oryzae* into four categories based on the cultural character; black with suppressed growth, black with cottony growth, black with fluffy growth and White with cottony growth. This was the first report by Kumar *et al*., (2011) on the black colony character of *B. oryzae* [5]. Kumari *et al.,* (2015) categorized 52 isolates into 5 groups: Black with fluffy growth, Black with suppressed growth, Grey with cottony growth, Grey and white mix with cottony growth and white with cottony growth [7]. Valarmathi and Ladhalakshmi (2018) characterized the brown spot pathogen into four groups: black with fluffy growth, grey with fluffy growth and white spots, grey with fluffy growth and grey with suppressed growth [13]. Manamgoda *et al.,* (2014) reported that *B, oryzae* produces conidiophores arising singly or in groups, brown to black, branched or simple, multi-septate. Conidia usually curved, rarely straight, navicular, fusiform, ob-clavate or almost cylindrical, hyaline when immature, becoming slightly brown when mature and germinating at both ends. Hilum was minute with slightly protruding [8]. On *in vitro* and *in vivo* studies, twelve fungicides were tested. The fungicides, Hexaconazole 5% EC and Tebuconazole 25% + Trifloxystrobin 50% WP and Zineb 68% + Hexaconazole 25% WG were effective in all concentration compared control in lab and field condition. Sunder *et al*., (2010) tested the efficacy of six fungicides against rice brown spot pathogen *in vivo* condition. Among the six fungicides, Propiconazole @ 1ml/l and Hexaconazole @ 2ml/l were effective and reduced the disease severity from 22.34% to 5.19% [11]. Gupta *et al*., (2013) tested the efficacy of seven fungicides at 0.1 % concentration. In this study, Propiconazole @ 0.1% concentration showed 97.89% inhibition over the control [2]. Kumar *et al.,* (2017) evaluated the efficacy of different fungicides combination against the brown spot pathogen in field and laboratory condition. Under i*n vitro* condition, Propiconazole @ 500 ppm showed 96.58 per cent inhibition over the control against the mycelial growth of brown spot pathogen, whereas under *in vivo* condition foliar spray of Propiconazole @ 1ml/l led to a significant reduction in disease severity to 37.26% and increase in the grain yield up to 55.49% [6].

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