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In Vitro Evaluation of Fungicides, Plant Extracts and Biocontrol Agents against Brown Leaf Spot of Paddy

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A B S T R A C T

Effect of fungicides, plant extracts and biocontrol agents against brown leaf spot of paddy were evaluated under laboratory conditions at Department of Plant Pathology, College of Agriculture, V. C. Farm, Mandya, Karnataka, during 2015-16. The fungicides were evaluated at 5 different concentrations *viz.*, 50, 100, 150, 200 and 250 ppm using poison food technique. The results revealed that, propiconazole25% EC to be statistically significant over the other fungicides tested, with 72.05% mycelia growth inhibition at 50 ppm concentration, and 100% mycelial growth inhibition at 100, 150, 200, and 250ppm concentrations. However hexaconazole 5% SC inhibited 72.95% and 74.23% mycelial growth at 50 and 100 ppm respectively, however, 100% mycelial growth inhibition was recorded at 150, 200 and 250 ppm concentration, respectively. All the nine plant extracts tested suppressed the mycelial growth of the pathogen. However, garlic clove extract proved to be the best which recorded 100% mycelial growth at all the 3 concentrations tested. This was followed by the marigold leaf extract which recorded 82.24, 85.55 and 88.16% mycelial growth inhibition at 5, 10 and 15 per cent concentration, respectively. Dual culture technique was used to evaluate the antagonistic activities of the bioagents. Maximum mycelial growth inhibition of 48.49% was recorded by *Trichoderma viride* whereas, *T. harzianum* and *Pseudomonas fluorescens* recorded 40.76% and 27.57% inhibition, respectively.

Introduction

Brown leaf spot of rice causes considerable qualitative and quantitative losses in rice growing countries of Asia, America and Africa (Ou, 1985). In India, the disease is widespread and known to cause4.6-29.0% losses in grain yield (Bedi and Gill, 1960).The pathogen has been found to cause stalk rot in addition to leaf spot and grain discolouration in non-scented high yielding varieties of rice in Haryana (Sunder *et al.,* 2005). The earlier recommended fungicides namely mancozeb and zineb have been found to impart insufficient control of the disease (Sunder *et al.,* 2010).Several plant derived products have been found inhibitory to mycelial growth and spore germination of the pathogen (Bisht and Khulbe, 1995; Amadioha, 2002; Kumar, 2006).

The antagonistic behavior of biocontrol agent like *Trichoderma harzianum* and other species *in vitro* resulted in the overgrowth of *B.*

oryzae by *T. harzianum.* While, the antifungal metabolites of *T. harzianum* completely prevented the linear growth of the pathogen (Abdel-Fattah *et al.*, 2007). Keeping this in view, the present study was undertaken to evaluate the efficacy of fungicides, botanicals and biocontrol agents against brown spot of rice under laboratory conditions.

Materials and Methods

In vitro experiments were conducted in the Department of Plant Pathology, College of Agriculture, V. C. Farm, Mandya during 2015-16.

Collection of diseased specimen and isolation of pathogen

The infected leaves showing typical brown leaf spot symptoms were collected from naturally infected paddy plants from the field in and around College of Agriculture, V. C. farm, Mandya, Karnataka. These leaves were properly packed in polythene covers and stored at 4°C for further studies. The infected portions of the leaves were used for isolation of the pathogen on potato dextrose agar medium. The pure culture of the pathogen was obtained by 'Separate Locate Isolate technique'. This culture was used for further laboratory studies.

In vitro evaluation of fungicides

The efficacy of ten different fungicides were tested at 50,100, 150, 200, 250 ppm, concentrations against the pathogen. The fungicides tested are listed in Table 1. These fungicides were evaluated by "Poison Food Technique" as given by Grover and Moore (1962). Required quantity of individual fungicide was added to cooled potato dextrose agar so as to get the desired concentration of the fungicide. Later, 20 ml of the poisoned medium was poured into Petri plates. Mycelial disc of 5 mm in diameter was placed at the centre of the agar plate. The medium without any fungicide served as control. Three replications were maintained for each concentration. Inoculated plates were incubated at 26±2°C and colony diameter was measured till the mycelial growth covered the entire Petri plates at 24 hr interval. The efficacy of the fungicides was expressed as per cent inhibition of mycelial growth, which was calculated by using the formula given by Vincent (1947).

$$I = \frac{C-T}{C} \times 100$$

Where, I = per cent inhibition, C = growth in control, T = growth in treatment

In vitro evaluation of plant extracts against the pathogen

Different parts of nine plant species *viz.*, onion bulb, garlic clove and leaves of lantana, marigold, datura, tulasi, eupatorium, parthenium and neem were used in this study (Table 2). The extract was obtained by crushing the tissues in distilled water at 1:1 w/v proportion.

The extract was filtered through double layered muslin cloth and later through Whatman No. 1 filter paper to get a clear filtrate. This filtrate was used as a stock solution for the study. The plant extracts were tested at 3 concentrations (5, 10 and 15%) on potato dextrose agar medium. Poison food technique was followed to evaluate the antifungal activity of the plant extracts as mentioned earlier. Observations were recorded at five days after inoculation. Radial growth of the fungus was measured and per cent inhibition of mycelial growth was calculated using the formula given by Vincent (1947) as mentioned above.

In vitro evaluation of bioagents against the pathogen

In vitro evaluation of four bioagents (Table 3) against *D. oryzae* was carried out using dual culture technique. Mycelial disc of the test fungus was inoculated at one end of the Petri plate and antagonistic fungus at the opposite end. In case of bacterial antagonist two mycelial disc of the pathogen were inoculated at the periphery of the Petri Plate and bacterial antagonist was streaked in the center of the same plate. Four replications were maintained for each treatment. The plates were incubated at 28°C. The radial growth of the pathogen was measured. Zone of inhibition was recorded by measuring the clear distance between the margin of the test fungus and antagonistic organism. The per cent inhibition of growth of the pathogen was calculated by using the formula suggested by Vincent (1947). Analysis of the experimental data was done by using completely randomised design (CRD) for the laboratory studies as suggested by Panse and Sukathme (1985).

Results and Discussion

In vitro evaluation of fungicide molecules

Among the different fungicides tested, propiconazole (25% EC) was significantly superior over the other fungicides. However it was on par with hexaconazole (5% SC), where both the fungicides recorded 100% mycelial growth inhibition at 100, 150, 200 and 250 ppm concentrations. Also that propiconazole (25% EC) recorded 72.07% average mycelial growth inhibition which was on par with hexaconazole (5% SC) (72.95%) at 50 ppm concentration. The results of present investigation are in conformity with the findings of Gupta *et al.,* (2013) wherein they reported that propiconazole (25% EC) was most effective in inhibiting the mycelial growth of *B. oryzae* with per cent inhibition of 97.89, 92.14, 91.92, 91.90 and 90.87% at 250, 200, 150, 100 and 50 ppm concentrations, respectively (Table 1). Minimum per cent inhibition of (29.65, 32.87 and 38.84%) mycelial growth was recorded in kresoxim methyl (44.3% SC) at 150, 200 and 250 ppm respectively, hence propiconazole (25% EC) and hexaconazole (5% SC) is found to be effective over the other chemicals.

In vitro evaluation of plant extracts against the pathogen

All the nine plant extracts tested suppressed the mycelial growth of the pathogen. The mycelial growth was reduced by 100% by garlic clove extract at all the 3 concentrations. The results are in accordance with Ahmed *et al.,* (2002) and Ahmed *et al.,* (2011), where the results obtained by Ahmed *et al.,* (2012) reported 91.7% average mycelial growth inhibition of *B. oryzae* by garlic clove extract proving to be effective against the pathogen. This was followed by the marigold leaf extract which recorded 82.24, 85.55 and 88.16% mycelial growth inhibition at 5, 10 and 15 per cent concentration, respectively. Least per cent growth inhibition of 2.36, 5.71 and 10.0% was exhibited by eupatorium leaf extract followed by lantana leaf extract 4.73, 7.10 and 16.56% growth inhibition at 5, 10 and 15 per cent concentration, respectively. Similar results on marigold leaf extract were reported by Islam *et al.,* (2015) on *Bipolaris sorokiniana* with 73.74 per cent inhibition of mycelial growth at 10 per cent concentration (Table 2).

In vitro evaluation of bioagents against the pathogen

Antagonistic activities of four bio-agents *viz., Trichoderma viride, T. harzianum, Pseudomonas fluorescens* and *Bacillus subtilis* were evaluated against *Bipolaris oryzae.* The results are presented in Table 3.

Table.1 *In vitro* evaluation of fungicides against growth of *B. oryzae*

Sl. No.	Treatments	Per cent inhibition					Mean
		Concentration (ppm)					
		50	100	150	200	250	
1	Tricyclozole (75% WP)	30.92 (33.78)	32.83 (34.95)	35.30 (36.45)	38.57 (38.39)	42.13 (40.47)	39.95 (35.06)
2	Benomyl (50% WP)	38.14 (38.13)	40.78 (39.68)	44.78 (42.00)	48.13 (43.92)	49.11 (44.49)	44.18 (39.94)
3	Carbendazium (50% WP)	35.31 (36.45)	38.22 (38.18)	42.08 (40.44)	46.74 (43.12)	49.84 (44.90)	42.43 (38.36)
4	Mancozeb (75% WP)	51.09 (45.62)	57.46 (49.28)	59.67 (50.57)	65.28 (53.89)	70.32 (56.98)	60.76 (48.49)
5	Propineb (70% WP)	42.48 (40.67)	46.18 (42.80)	48.93 (44.38)	52.98 (46.70)	54.85 (47.78)	49.10 (42.62)
6	Tebuconazole (25.9% EC)	58.06 (49.63)	58.39 (49.82)	67.02 (54.94)	74.73 (59.82)	75.48 (60.31)	66.70 (51.46)
7	Propiconazole (25% EC)	72.05 (58.08)	100.00 (89.99)*	100.00 (89.99)	100.00 (89.99)	100.00 (89.99)	94.42 (79.35)
8	Hexaconazole (5% SC)	72.95 (58.65)	74.23 (59.48)	100.00 (89.99)	100.00 (89.99)	100.00 (89.99)	89.36 (69.38)
9	Kresoxim methyl (44.3% SC)	22.60 (28.35)	25.58 (30.38)	29.65 (32.99)	32.87 (34.98)	38.84 (38.54)	29.90 (30.58)
10	Azoxystrobin (23% SC)	34.48 (35.96)	38.88 (38.57)	40.35 (39.43)	42.42 (40.63)	46.49 (42.98)	40.52 (37.99)
		Fungicides (F)		**Concentration (C)**		**F X C**	
	SEm(±)	**0.18**		**0.25**		**0.53**	
	CD@1%	**0.31**		**0.47**		**1.05**	
	CV%	**0.87**					

*Figures in parenthesis are arcsine transformed values

Table.2 Effect of different concentrations plant extracts against growth of *B. oryzae* under *in vitro* conditions

Sl. No.	Treatments	Per cent growth inhibition			Mean
		Concentrations (%)			
		5	10	15	
1	Onion (*Allium cepa)*	76.33 (60.87)	76.52 (60.88)	78.30 (62.17)	76.95 (61.37)
2	Garlic (*Allium sativum)*	100.0 (90.00)	100.0 (90.00)	100.0 (90.00)	100.00 (90.00)
3	Lantana (*Lantana camara)*	04.73 (12.52)	07.10 (15.46)	16.56 (23.82)	9.37 (17.34)
4	Marigold (*Tegeteserecta)*	82.29 (65.32)	85.55 (69.80)	88.16 (69.75)	86.23 (68.38)
5	Dathura (*Daturastramonium)*	23.07 (28.71)	33.39 (35.20)	35.50 (36.51)	30.57 (33.51)
6	Tulasi (*Ocimumsantum*)	70.41 (57.19)	74.55 (59.99)	74.55 (59.91)	73.45 (58.81)
7	Eupatorium (*Eupatorium rugosum)*	02.36 (9.07)	05.71 (13.65)	10.0 (18.49)	6.04 (13.70)
8	Parthenium (*Partheniumhisteroporus)*	70.41 (57.30)	73.30 (58.96)	79.30 (59.80)	72.98 (58.81)
9	Neem (*Azadirachtaindica*)	73.96 (58.97)	75.93 (60.33)	78.69 (62.19)	75.72 (60.32)
		Plant extracts (P)	**Concentrations (C)**		**P X C**
	Sem (±)	**0.11**	**0.12**		**0.48**
	CD@1%	**0.24**	**0.43**		**0.76**
	CV (%)	**0.56**			

*Figures in parenthesis are arcsine transformed values

Table.3 *In vitro* evaluation of different bioagents against *B. oryzae*

Sl. No.	Bioagents	Mean radial growth (mm)	Per cent growth inhibition
1	*Trichoderma viride*	32.33	48.49 (44.13)
2	*Trichoderma harzianum*	35.33	40.76 (39.67)
3	*Pseudomonas fluorescens*	43.67	27.57 (31.67)
4	*Bacillus subtilis*	47.83	17.77 (24.92)
5	Control	59.66	
	Sem (±)	**0.33**	**0.51**
	CD@1%	**1.06**	**1.65**
	CV (%)	**1.45**	**2.66**

*Figures in parenthesis are arcsine transformed values

The results, obtained revealed maximum inhibition of mycelial growth by *T. viride* (48.49%) win comparison with other bioagents. Whereas, *T. harzianum* and *P. fluorescens* recorded 40.76% and 27.57% inhibition, respectively. Least per cent inhibition of 17.77% mycelial growth was recorded by *B. subtilis*. The present studies are in conformity with the observations made by Abdel-Fattah *et al.*, (2007), wherein they reported that *T. harzianum* had better antagonistic activity thereby suppressing 83.33% mycelial growth of *B. oryzae*. Khalili *et al.*, (2012) also recorded *Trichoderma* isolates significantly inhibiting the mycelial growth of the pathogen (69%) under *in vitro* by producing volatile and non-volatile metabolites.

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