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In vitro Efficacy of Fungicides, Botanicals and Bioagents against Brown Leaf Spot of Rice Caused by *Bipolaris oryzae*

Meghana S. Nayak* and Hiremath S. V.

Department of Plant Pathology, College of Agriculture, Dharwad

University of Agricultural Sciences, Dharwad-580005, Karnataka, India

*Corresponding Author E-mail: meghananayak.1600@gmail.com

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ABSTRACT

Effect of fungicides, botanicals and biocontrol agents against brown leaf spot of paddy caused by Bipolaris oryzae were evaluated under laboratory conditions at Department of Plant Pathology, College of Agriculture, Dharwad, Karnataka during 2018-19. The fungicides were evaluated at different concentrations using poison food technique. Systemic fungicides were evaluated at 0.05, 0.1 and 0.15, non-systemic fungicides at 0.1, 0.2 and 0.3, combi products at 0.05, 0.1 and 0.2 and botanicals were evaluated at 5, 7.5 and 10 per cent concentration respectively with four replications. The results revealed that, the maximum mean per cent of mycelial inhibition was observed in captan 50 % WP (73.98 %), propiconzole 25 % EC (100 %) and tebuconazole 50 % + trifloxystrobin 25 % 75 WG (100 %) in non systemic, systemic and combi products, respectively. The mean mycelia inhibition increases with increasing in concentration of fungicides. Among the botanicals crude neem oil (32.55 %) gave maximum mean per cent of mycelial inhibition which was significantly superior to other treatments. The least mean per cent of inhibition of the fungus was recorded in ginger rhizome extract (6.53 %). Dual culture technique was used to evaluate the antagonistic activities of the bioagents maximum mycelial inhibition (62.75 %) followed by Bacillus subtilis (51.76 %). Least mycelial inhibition was observed with fungal antagonistic organism Trichoderma harzianum (27.06 %)

Key words: *In vitro, Fungicides, Botanicals, Bio-agents, Inhibition*

INTRODUCTION

Rice (*Oryza sativa* L.) is the staple and important food crop consumed over half of the world population. It is a nutritious cereal crop, provides 20 per cent of the calories, 15 per cent of protein, carbohydrate, minerals and fibres. Diseases which cause heavy yield loss are brown spot, leaf blast, bacterial blight, sheath blight and tungro virus. Among the fungal diseases of rice, brown spot is a historically important disease. Brown leaf spot of rice caused by *Bipolaris oryzae* Subr. and Jain (=*Helminthosporium oryzae* teleomorph = *Cochliobolus miyabeanus*) is known to occur in Japan since 1900.

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It is also called as 'nai-yake' *i.e.* seedling blight, sesame leaf spot and *Helminthosporiosis.* In India, brown leaf spot of rice is known to occur in all the rice growing state[1,6]. and was first reported from Madras in 1922 by Sundraraman. The disease is of great importance in several countries and has been reported to cause enormous losses in grain yield (upto 90 %) particularly when leaf-spotting phase assumes epiphytotic proportions as observed in Great Bengal Famine during 1942[2].

MATERIAL AND METHODS

In vitro evaluation of fungicides, botanicals and biocontrol agents against *Bipolaris oryzae* were conducted in the Department of Plant Pathology, College of Agriculture, University of Agricultural Sciences, Dharwad.

Collection of diseased sample and isolation of pathogen

The infected leaves showing typical brown leaf spot symptoms were collected from different locations. 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 hyphal tip method. This culture was used for different laboratory studies.

***In vitro* evaluation of fungicides and botanicals**

Poisoned food technique was followed to test the efficacy of six of each non-systemic, systemic and combi products and four botanicals against *B. oryzae*. Systemic fungicides were evaluated at 0.05, 0.1 and 0.15 per cent concentration, non-systemic fungicides were tested at 0.1, 0.2 and 0.3 per cent concentration, combi products were evaluated at 0.05, 0.1 and 0.2 per cent concentration and botanicals were evaluated at 5, 7.5 and 10 per cent concentration with four replications. The pathogen was grown on PDA medium in petri plates for ten days prior to setting the experiment.

Fungicide suspension was prepared in PDA by adding required quantity of fungicides concentration. Similarly botanicals were crushed and filtered through muslin cloth and required quantity of extract is added to PDA and then sterilization was done. Twenty ml of poisoned medium was poured in each of the sterilized petri plates. Mycelial disc of 7 mm was taken from the periphery of seven days old culture and placed in the center and incubated at 27 ± 1 °C till growth of the fungus touched the periphery in the control plate. Suitable checks were maintained without the addition of any fungicide, three replications were maintained for each treatment. The colony diameter of the fungus was measured in two directions and average was worked out. The per cent inhibition of growth was calculated by using the formula given by Vincent[10].

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

Where,

I = Per cent inhibition of mycelium

C = Growth of mycelium in control

T = Growth of mycelium in treatment

***In vitro* evaluation of bioagents**

The antagonistic microorganisms like *Trichoderma harzianum, Pseudomonas fluorescens* and *Bacillus subtilis* were evaluated for their antagonistic effect under *in vitro* conditions against *B. oryzae* by 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[10]. Analysis of the experimental data was done by using completely randomised design (CRD)

for the laboratory studies as suggested by Panse and Sukathme[7].

RESULTS AND DISCUSSION

In vitro evaluation of fungicide molecules

Among the non systemic fungicides evaluated, maximum mean per cent of mycelial inhibition was observed in captan 50 % WP (73.98 %) which was on par with copper oxychloride 50 % WP (73.20 %). The least mean per cent of inhibition of fungus was recorded in chlorothalonil 25 % WP (21.83 %) (Table 1, Fig 1). Among six systemic fungicides evaluated, maximum mean per cent of mycelial inhibition was recorded in propiconzole 25 % EC (100 %) which was significantly superior to all other fungicides followed by myclobutanil 10 % WP (93.72 %). The least mean per cent of inhibition was recorded in azoxystrobin 25 % SC (13.59 %) (Table 2, Fig 1). Among six combi product fungicides evaluated, cent mean per cent of mycelial inhibition was recorded in tebuconazole 50 % + trifloxystrobin 25 % 75 WG (100 %) which was significantly superior to all other fungicides followed by captan 70 % + hexaconazole 5 % 75 WP (96.60 %). The least mean per cent of mycelia inhibition was recorded in mancozeb 50 % + carbendazim 25 % 75 WS (22.48 %) (Table 3, Fig 1).

The results of present investigation are in conformity with the findings of Gupta *et al*.[3], where they reported that propiconazole (25 % EC) was most effective in inhibiting the mycelial growth of *D. oryzae* with per cent inhibition of 97.89 at 250 ppm concentration and with Hunjan *et al*.[4], who tested systemic and combi fungicides against *D. oryzae* and reported that among the combi fungicides tebuconazole 50 % + trifloxystrobin 25 % WG (nativo 75 % WG) completely inhibited the mycelial growth of the *D. oryzae.*

In vitro evaluation of botanicals

The results revealed that the inhibition of mycelial growth varied significantly with different treatments. Among the four botanicals evaluated, crude neem oil (32.55 %) gave maximum mean per cent of mycelia inhibition which was significantly superior to other treatments. The least mean per cent of inhibition of the fungus was recorded in ginger rhizome extract (6.53 %) (Table 4, Fig 2). These results are in conformity with Kumar *et al*.[5], who tested the efficacy of neem oil and neem leaf extract against *H. oryzae* by poison food technique and recorded that neem oil at 3 per cent concentration showed highest inhibition growth of pathogen (54.75 %) followed by neem leaf extract at 7 per cent (53.88 %).

In vitro evaluation of bioagents

Results clearly indicated that, all antagonistic bioagents significantly suppressed the growth of *B. oryzae*. However, *Pseudomonas fluorescens* registered significantly maximum mycelial inhibition (62.75 %) followed by *Bacillus subtilis* (51.76 %). Least mycelial inhibition was observed with fungal antagonistic organism *Trichoderma harzianum* (27.06 %) (Table 5, Fig 3). The lower mycelial growth of the pathogen may be due to antibiotics produced by the bio control agents. Some of the fluorescent pseudomonads have currently received world-wide attention due to the production of a wide range of antimicrobial compounds antibiotics such as phenazine-1-carboxylic acid, pyoluteorin phenazine-1-carboxamide, viscosinamide and tesin, 2,4- diacetylphloroglucinol (DAPG)[8].

Table 1: *In vitro* evaluation of non systemic fungicides against *Bipolaris oryzae*

Sl.No.	Fungicide	Per cent mycelial inhibition			Mean
		Concentration (%)			
		0.10	0.20	0.30	
1	Copper oxychloride 50 % WP	63.53 (52.85) *	67.84 (55.45)	88.24 (69.96)	73.20 (59.42)
2	Mancozeb 75 % WP	55.69 (48.27)	65.49 (54.02)	73.33 (58.91)	64.83 (53.73)
3	Chlorothalonil 75 % WP	15.69 (23.33)	20.00 (26.56)	29.80 (33.09)	21.83 (27.66)

4	Captan 50 % WP	63.53 (52.85)	69.41 (56.42)	89.02 (70.69)	73.98 (59.98)
5	Zineb 70 % WP	20.00 (26.56)	31.76 (34.30)	44.71 (41.96)	32.15 (34.27)
6	Propineb 70 % WP	40.00 (39.23)	46.27 (42.86)	58.82 (50.08)	48.36 (44.05)
	Mean	43.07 (40.51)	50.12 (44.93)	63.98 (54.11)	
		Fungicide (A)	Concentration (B)	A × B	
S.Em.±		0.23	0.15	0.40	
C.D. at 1 %		0.87	0.57	1.52	

* Arcsine transformed values

Table 2: *In vitro* evaluation of systemic fungicides against *Bipolaris oryzae*

Sl. No.	Fungicide	Per cent mycelial inhibition			Mean
		Concentration (%)			
		0.05	**0.10**	**0.15**	
1	Propiconazole 25 % EC	100.00 (90.00) *	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
2	Pyraclostrobin 20 % WG	30.59 (33.58)	45.88 (42.64)	57.65 (49.40)	44.70 (41.87)
3	Tebuconazole 25 % EC	87.45 (69.26)	90.98 (72.53)	94.51 (76.47)	90.98 (72.75)
4	Thiophenate methyl 70 % WP	5.49 (13.53)	10.20 (18.61)	34.51 (35.98)	16.73 (22.70)
5	Myclobutanil 10 % WP	90.59 (72.16)	93.73 (75.51)	96.86 (79.84)	93.72 (75.83)
6	Azoxystrobin 25 % SC	7.06 (15.37)	10.59 (18.97)	23.14 (28.75)	13.59 (21.03)
	Mean	53.53 (48.98)	71.89 (53.04)	67.77 (60.07)	
		Fungicide(A)	Concentration (B)	A × B	
S.Em. ±		0.24	0.16	0.41	
C.D. at 1 %		0.91	0.60	1.58	

* Arcsine transformed values

Table 3: *In vitro* evaluation of combi products against *Bipolaris oryzae*

Sl. No.	Fungicide	Per cent mycelial inhibition			Mean
		Concentration (%)			
		0.05	**0.1**	**0.2**	
1	Azoxystrobin 18.2 % w/w + Difenconazole 11.4 % w/w SC	87.84 (69.60) *	90.59 (72.16)	94.51 (76.47)	90.98 (72.74)
2	Tebuconazole 50 % + Trifloxystrobin 25 % WG	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
3	Flusilazole 12.5 % + Carbendazim 25 % SC	80.00 (63.44)	87.45 (69.26)	92.55 (74.17)	86.66 (68.95)
4	Captan 70 % + Hexaconazole 5 % 75 WP	94.12 (76.01)	95.69 (78.04)	100.00 (90.00)	96.60 (81.35)
5	Mancozeb 50 % + Carbendazim 25% WS	11.76 (20.04)	23.53 (29.01)	32.16 (34.54)	22.48 (27.86)
6	Azoxystrobin 11 % + Tebuconazole 18.3 % w/w SC	75.29 (60.20)	83.14 (65.76)	90.98 (72.53)	83.13 (66.16)
	Mean	74.83 (63.21)	80.06 (67.37)	85.03 (72.95)	
		Fungicide(A)	Concentration (B)	A × B	
S.Em.±		0.25	0.16	0.42	
C.D. at 1 %		0.94	0.61	1.62	

* Arcsine transformed values

Table 4: *In vitro* evaluation of botanicals against *Bipolaris oryzae*

Sl. No.	Botanicals	Per cent mycelial inhibition			Mean
		Concentration (%)			
		5.0	7.5	10	
1	Garlic bulb extract	3.92 (11.40) *	7.65 (16.05)	16.08 (23.64)	9.21 (17.03)
2	Neem leaf extract	4.71 (12.46)	8.24 (16.65)	20.00 (26.56)	10.98 (18.55)
3	Crude neem oil (300 ppm)	15.29 (23.01)	33.73 (35.50)	48.63 (44.21)	32.55 (34.24)
4	Ginger rhizome extract	2.94 (9.84)	6.86 (15.18)	9.80 (18.24)	6.53 (14.42)
	Mean	6.71 (14.17)	14.12 (20.84)	23.62 (28.16)	
		Fungicide (A)	Concentration (B)	A × B	
S.Em.±		0.22	0.14	0.38	
C.D. at 1 %		0.84	0.55	1.46	

* Arcsine transformed values

Table 5: *In vitro* evaluation of bioagents against *Bipolaris oryzae*

Sl. No.	Bioagents	Per cent inhibition of mycelial growth
1	*Trichoderma harzianum*	27.06 (31.33) *
2	*Pseudomonas fluorescens*	62.75 (52.37)
3	*Bacillus subtilis*	51.76 (45.99)
	Mean	47.19 (43.23)
	S.Em. ±	0.49
	C.D. at 1 %	2.57

* Arcsine transformed values

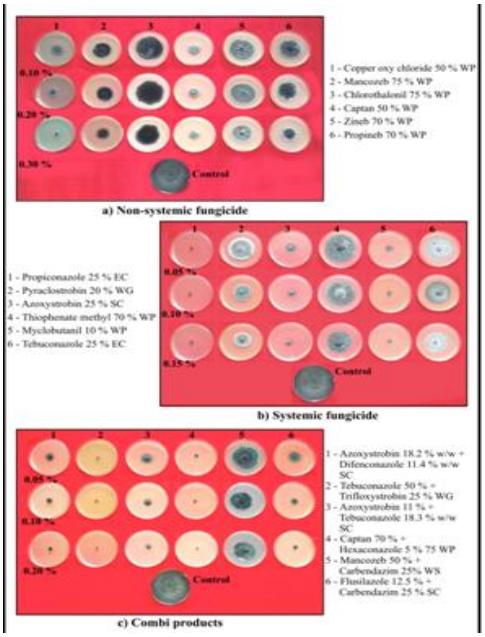

Fig. 1: *In vitro* efficacy of fungicides against *Bipolaris oryzae*

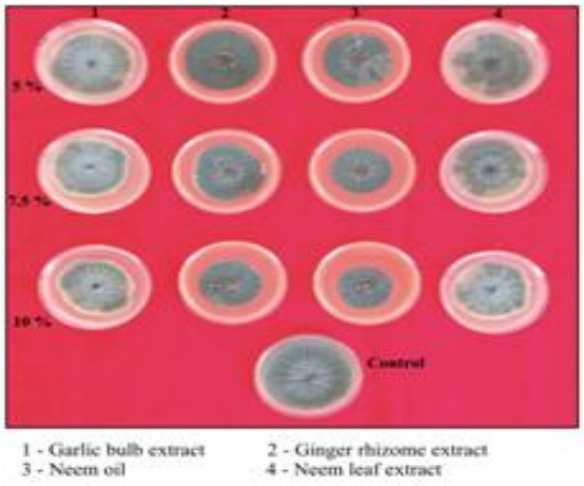

Fig. 2: *In vitro* efficacy of botanicals against *Bipolaris oryzae*

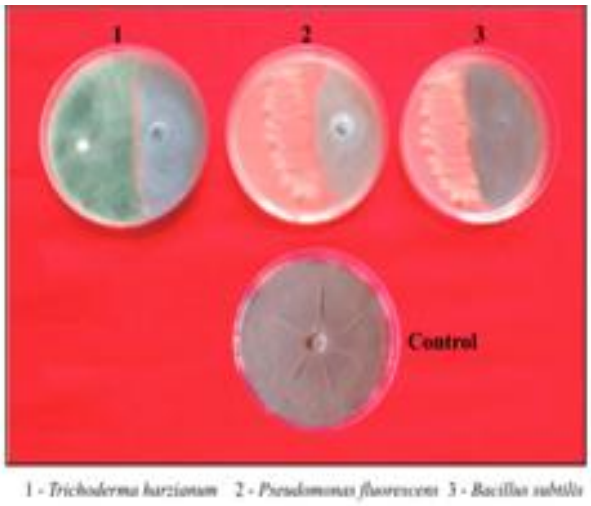

Fig. 3: *In vitro* efficacy of botanicals against *Bipolaris oryzae*

REFERENCES

1. Gangopadhyay, S., Current Concepts on Fungal Diseases of Rice. Today and tomorrow's Printers and Publishers, New Delhi, pp. 349 (1983).
2. Ghose, R. L. M., Ghatge, M. B. and Subramanian, V., Rice in India (revised edn.), ICAR, New Delhi, p. 474 (1960).
3. Gupta, V., Shamas, N., Razdan, V. K., Sharma, B. C., Sharma, R., Kaur, K., Singh, I., John, J. and Kumar, A., Foliar application of fungicides for the management of brown spot disease in rice (*Oryza sativa* L.) caused by *Bipolaris oryzae*. *African J. Agric. Res.*, **8(25):** 3303-3309 (2013).
4. Hunjan, M. S., Lore, J. S., Pannu, P. P. S. and Thind, T. S., Performance of some new fungicides against sheath blight and brown spot of rice, *Pl. Dis. Res.*, **26(1):** 61-67 (2011).
5. Kumar, H., Ahmad, S. and Zacharia, S., Efficacy of fungal, bacterial bioagents and botanicals against Brown spot (*Helmintlhosporium Oryzae*) of rice (*Oryzae Sativa). Res. J. Chem. Environ. Sci*., **3(2):** 27-31 (2015).
6. Ou, S. H., Rice diseases (Second Edn), Kew, Commonwealth Mycological Institute, Hyderabad (India), p. 380 (1985).
7. Panse, V. G. and Sukhatme, P. V., Statistical Methods for Agricultural Workers, ICAR Publications, New Delhi, India, p.143 (1978).
8. Shanahan, P., O'Sullivan, D. J., Simpson, P., Glennon, J. D. and O'Gara, F., Isolation of 2,4- diacetylphloroglucinol

from a fluorescent pseudomonad and investigation of physiological parameters influencing its production. *Appl. Environ. Microbiol.*, **58:** 353-358 (1992).

9. Sundararaman, S., *Helminthosporium* disease of rice. *Bull. Agril. Res. Inst.*, Pusa, **128:** 1-7 (1922).
10. Vincent, J. M., The esters of 4-hydroxy benzoic acid and related compounds. Methods for the study of their fungi static properties. *J. Soc. Chem. Ind. London*., **16:** 746-755 (1947).