International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2018; 6(2): 1875-1879

Received: 04-01-2018
Accepted: 06-02-2018

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Evaluation of fungicides, bio-agents and plant extracts against *Pyricularia oryzae*

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Abstract
Rice is the major source of food for more than half of the world population. Rice suffers from many biotic and abiotic factors which results in the lower productivity. Blast disease is considered as major threat to rice cultivation. Thus, 7 fungicides, 4 bio agent and 7 plant extracts were evaluated. Among fungicides tested, all the fungicides found effective but Carbendazim and Propiconazole showed inhibition of 84.07 per cent and 74.25 per cent respectively at 500 ppm. Among the bio-agents tested, *Trichoderma viride* showed maximum inhibition with 64.44 per cent followed by *Trichoderma harzianum* with inhibition of 59.04 per cent, the least inhibition was recorded in *Bacillus subtilis* with inhibition of 47.48 per cent. The extracts of *Lantana camara* (55.80%), Neem (53.06%) and Nilgiri (51.83%) were showed maximum inhibition of pathogen at higher concentration.

Keywords: *In vitro*, Rice, fungicides, plant extract, bio-agents, *Pyricularia oryzae*

Introduction
Rice (*Oryza sativa* L.) is an important cereal crop belonging to the grass family Poaceae. Nutritionally rice has calorific value of 363 K Cal which is higher than any other cereal crop with easily digestible carbohydrates (80.4%) and high quality protein (6.7%) possessing biological value due to high content of essential amino acid. The world's estimated rice production is 496.0 million metric tons during 2016 [1]. India is the largest rice growing country accounting for about one third of the world acreage under the crop. In India's annual rice production is 103.6 million tons during 2016 [1]. Rice is known to attack by 50 diseases including 6 bacterial, 21 fungal, 4 nematodes, 12 viral and 7 miscellaneous diseases and disorders[2] [3] [4]. However, major diseases are rice blast, brown spot, bacterial leaf blight and leaf streak, sheath blight, sheath rot, Fusarium wilt or Bakanae, stem rot, Tungro virus, false smut and post-harvest diseases [5]. Blast disease is considered as principle fungal disease of rice and it is major threat to rice production due to its distribution in all rice growing areas and its destructiveness under congenial conditions. It is distributed worldwide and prevailing in more than 85 countries of the world [6] [7]. Blast disease caused by *Pyricularia oryzae* Cavara [Synonym *Pyricularia grisea* Sacc., the anamorph of *Magnaporthe grisea* (T.T Hebert) Yaegashi and Udagawa]. The fungus can cause losses up to 90 per cent under favorable conditions. The pathogen attacks all the aerial parts of plants at any stage of crop growth right from germination to harvest. The disease occurs as leaf blast, node blast, neck or panicle blast. The neck or panicle blast is more damaging stage by the pathogen. The main objective of this study was to evaluate the effect of different fungicides, bio-agents and plant extracts against *Pyricularia oryzae.*

Material and Methods
The *in vitro* study was conducted at AHRS, Ponnampet, Karnataka during March 2017 to find out the effect of fungicides, bio-agents and plant extracts on mycelial growth of *Pyricularia oryzae.*

Effect of fungicides on growth of *Pyricularia oryzae*
Four systemic fungicides viz., Tricyclazole 75%WP, Carbendazim 50%WP, Hexaconazole 5% EC, Propiconazole 25%EC and three combi-products viz., Nativo 75%WG (Tebuconazole 50% + Trifloxystrobin 25%), ICF-110 (Tricyclazole 45% + Hexaconazole 10% WG)

and Tricyclazole 20% + Tebuconazole 16% SC were tested under laboratory condition at the concentration of 500 ppm and 1000 ppm by using poison food technique [8]. The concentrations of fungicides were added at the time of pouring sterilized Oat meal agar medium. OMA medium without any fungicide was served as control. Streptomycin sulphate at 1ml/l is added to the sterilized OMA medium before pouring into petri plates to avoid bacterial contaminations. After solidification, 5 mm disc from pure culture of fungus was placed in the centre of petri dishes and incubated at 27 ^{0}±1 ^{0}C. Three replications were maintained for each treatment. The radial growth of fungus was recorded when full growth in control plates was noticed and the per cent inhibition over control was calculated by using the formula given by [9].

$$I = \frac{(C - T)}{C} X\ 100$$

Where,
I= Per cent inhibition, C= Growth of the fungus in control, T= Growth of the fungus in treatment.

Effect of bio-agents on growth of *Pyricularia oryzae*

Bio-control agents such as *Pseudomonas fluorescens, Bacillus subtilis, Trichoderma viride,* and *Trichoderma harzianum* were tested by using dual culture technique under laboratory conditions against *Pyricularia oryzae.* Bio-agents and pathogen were inoculated side by side in a single Petri dish containing solidified OMA medium. Five replications were maintained for each treatment with one control by maintaining only pathogen. The plates were incubated for 7 days at 27 ^{0} ±1 ^{0}C. Observations on width of inhibition zone and mycelial growth of the pathogen was recorded in all treatments when complete growth of the pathogen was observed in control plate and per cent inhibition of growth of pathogen was calculated using formula given by [9].

Effect of plant extracts on growth of *Pyricularia oryzae*

Hundred gram of leaves of Neem, Pongamia, Eualyptus, *Lantana camara*, Marigold, Tulsi and cloves of garlic were washed with running tap water and surface sterilized with 70 per cent ethyl alcohol and rinsed with sterile distilled water 2 to 3 times. They were grinded with help of pestle mortar and were passed through muslin cloth by adding distill water. The plant extracts were tested at 5%, 10% and 15% concentrations. The plant extracts were added aseptically to sterilize OMA medium so as to get desired concentrations and poured into petridishes. Streptomycin sulphate at 1ml/l is added to the sterilized OMA medium to avoid bacterial contamination. The plant extracts were tested by using poison food technique. Three replications were maintained for each treatment. The radial growth of the colony was recorded when the maximum growth was obtained in control and per cent inhibition was calculated by using the formula given by [9].
Analysis and interpretation of the experimental data was done by using completely randomized design (CRD) and Factorial CRD for laboratory studies ANOVA [10, 11].

Results and Discussion

Effect of fungicides on growth of *Pyricularia oryzae*

The results indicated that, all the fungicides evaluated at two different concentrations were effective on inhibition of growth of the *Pyricularia oryzae* (Table 1, Plate 1 and Fig 1). Among systemic fungicides evaluated, cent percent inhibition was observed in Tricyclazole and Hexaconazole at all the concentrations but maximum growth of inhibition of pathogen was observed in Carbendazim (84.07%) at 500 ppm followed by Propiconazole (74.25%). At 1000 ppm, both Carbendazim and Propiconazole were on par with each other with inhibition of 91.10 per cent and 89.62 per cent. All the three combi-products *viz.*, Nativo, Tricyclazole 20% + Tebuconazole 16% SC and ICF-110 showed complete inhibition of 100% of *Pyricularia oryzae* at both the concentrations. The results obtained here are in conformity with the findings of [12] who also reported the complete inhibition of growth of *Pyricularia oryzae* in Nativo and Tricyclazole. Similarly, [13] also reported that Tricyclazole, Carbendazim, Hexaconazole as effective fungicides against *Pyricularia oryzae*.

Table 1: *In vitro* evaluation of fungicides on inhibition of mycelial growth of *Pyricularia oryzae*

S. No	Fungicide	Inhibition (%)		
		Concentration (ppm)		
		500	1000	Mean
1	Tricyclazole 75% WP	100.00 (90.00)*	100.00 (90.00)	100.00 (90.00)
2	Carbendazim 50% WP	84.07 (66.45)	91.10 (72.61)	87.58 (69.53)
3	Hexaconazole 5% EC	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
4	Propiconazole 25% EC	74.25 (59.48)	89.62 (71.17)	81.93 (65.32)
5	Tebuconazole 45% + Trifloxystrobin 30% WG	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
6	Tricyclazole 20% + Tebuconazole 16% SC	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
7	Tricyclazole 45% + Hexaconazole 10% WG	100.00 (90.00)	100.00 (90.00)	100.00 (90.00)
	Mean	94.04 (82.27)	97.24 (84.82)	95.64 (83.55)

	Fungicides (F)	Concentration (C)	F x C
S. Em±	0.12	0.03	0.24
CD at 1 %	0.47	0.11	0.94

*figures in parenthesis indicates arc sine transformed values

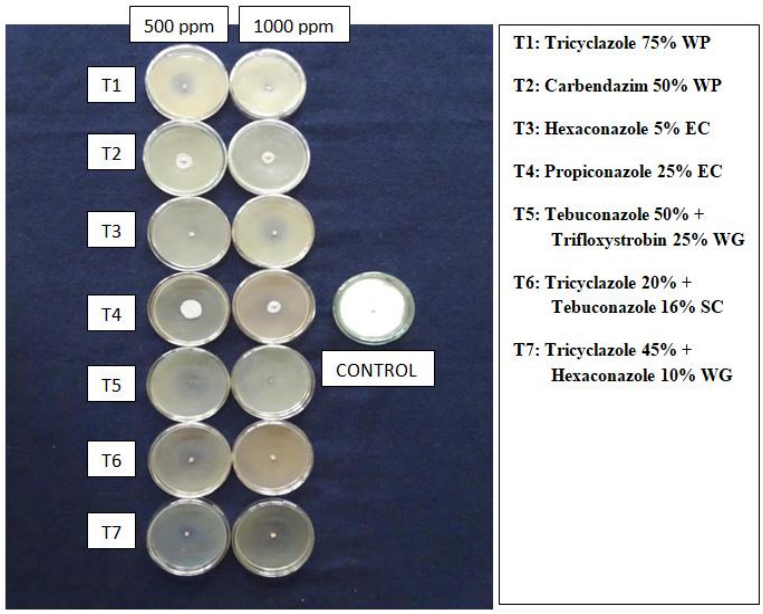

Plate 1: Effect of fungicides on inhibition of mycelial growth of *Pyricularia oryzae*

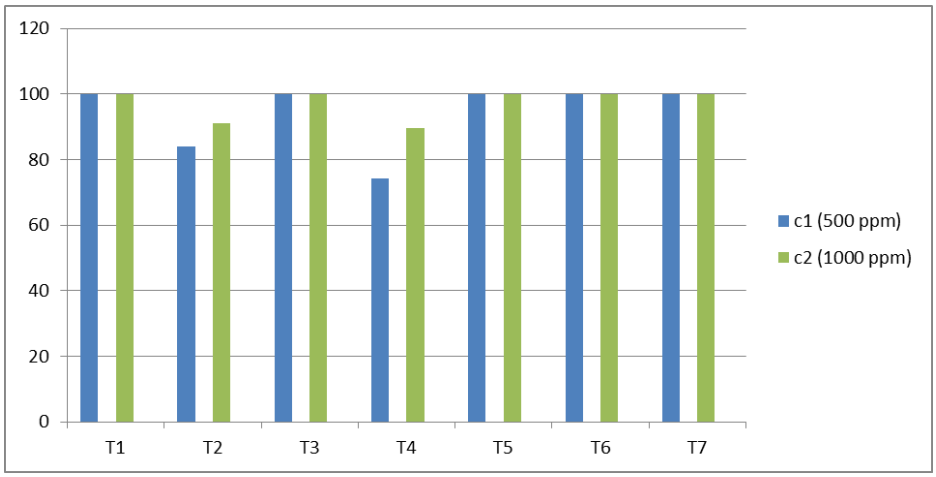

Fig 1: Effect of fungicides on inhibition of mycelial growth of *P. oryzae*

Effect of bio-agents on growth of *Pyricularia oryzae*

Four different bio-agents were evaluated for their efficacy on inhibition of growth of the pathogen**.** All the bio-agents tested were differed significantly with respect to the inhibition of growth of the pathogen (Table 2, Plate 2 and Fig 2). The maximum inhibition of growth of the pathogen was observed in *Trichoderma viride* with 64.44 per cent followed by *Trichoderma harzianum* with inhibition of 59.04 per cent followed by *Pseudomonas fluorescens* (52.48 %). The least inhibition of growth of the pathogen was observed in *Bacillus subtilis* with inhibition of 47.48 per cent. The similar results were obtained by [14] who reported the maximum inhibition of *Pyricularia oryzae* by *Pseudomonas fluorescens* (67.78%) and further stated that *Pseudomonas fluorescens* is ideal bio-agent to control *P.oryzae* because it controls the pathogen through mode of actions like antibiosis, competition for nutrients and space, production of siderophores and lytic enzymes. Similarly, [15] reported the maximum inhibition of growth of *P.oryzae* in *Trichoderma viride* (62%) and in *Trichoderma harzianum* up to the extent of 44%.

Table 2: *In vitro* evaluation of bio-agents on inhibition of mycelial growth of *Pyricularia oryzae*

S. No	Bio-agent	Inhibition (%)
1	*Pseudomonas fluorescens*	52.48 (46.44)*
2	*Trichoderma viride*	64.44 (53.42)
3	*Trichoderma harzianum*	59.04 (50.23)
4	*Bacillus subtilis*	47.48 (43.58)
	S. Em±	0.41
	CD at 1 %	1.70

*figures in parenthesis indicates arc sine transformed values

Plate 2: Effect of bio-agents on inhibition of mycelial growth of *P. oryzae*

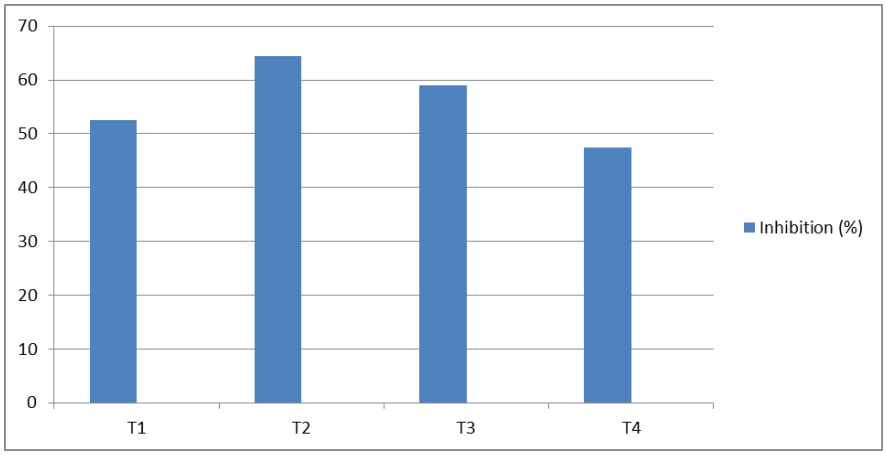

Fig 2: Effect of bio-agents on inhibition of mycelial growth of *P.oryzae*

Effect of plant extracts on growth of *Pyricularia oryzae*

Seven different plant extracts were tested for their efficacy on inhibition of growth *Pyricularia oryzae* at three different concentrations. Results indicated that, all the plant extracts differed with respect to inhibition of growth of *Pyricularia oryzae* (Table 3, Plate 3 and Fig 3).

Table 3. *In vitro* evaluation of botanicals on inhibition of mycelial growth of *Pyricularia oryzae*

S. No	Botanicals	Inhibition (%) Concentration (%)			
		5	10	15	Mean
1	Neem leaf extract	22.27 (28.17)	29.42 (32.86)	53.06 (46.77)*	34.91 (35.93)
2	Marigold leaf extract	21.12 (27.37)	25.32 (30.22)	39.67 (39.05)	28.70 (32.21)
3	Lantana leaf extract	26.15 (30.77)	36.93 (37.44)	55.80 (48.35)	39.62 (38.85)
4	Garlic clove extract	24.97 (29.99)	31.55 (34.19)	45.32 (42.33)	33.94 (35.50)
5	Pongamia leaf extract	19.99 (26.57)	23.91 (29.28)	28.26 (32.13)	24.05 (29.32)
6	Eucalyptus leaf extract	26.86 (31.23)	38.85 (38.57)	51.83 (46.07)	39.18 (38.62)
7	Tulsi leaf extract	21.23 (27.45)	26.60 (31.06)	30.07 (33.27)	25.96 (30.59)
	Mean	23.22 (28.79)	30.36 (33.37)	43.43 (41.13)	32.33 (34.43)

	Botanicals (B)	Concentration (C)	B x C
S. Em±	0.19	0.12	0.32
CD at 1 %	0.50	0.33	0.87

*figures in parenthesis indicates arc sine transformed values

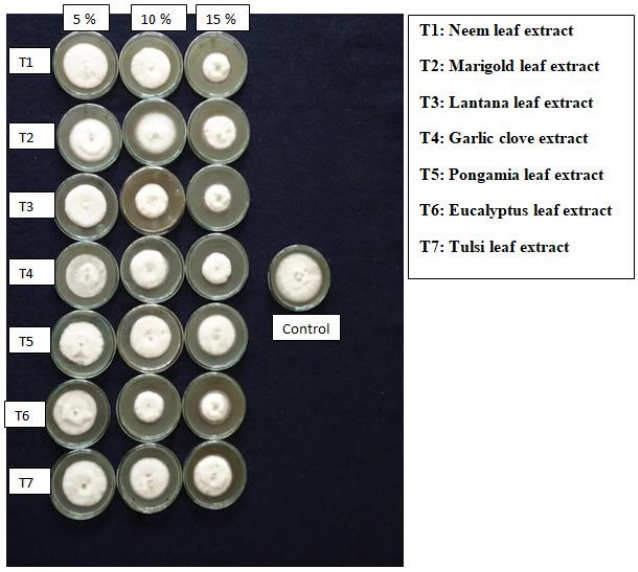

Plate 3: Effect of plant extracts on inhibition of mycelial growth of *Pyricularia oryzae*

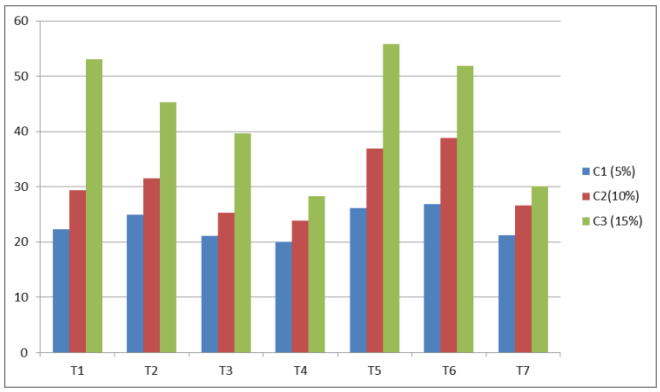

Fig 3: Effect of plant extracts on inhibition of mycelial growth of *P. oryzae*

Maximum inhibition of growth of the pathogen was observed in *Lantana camara* leaf extract with 55.80% at 15% concentration followed by Neem (53.06%) and Eucalyptus (51.83%) at same concentration. As the concentrations increased from 5 to 15 per cent, inhibition of growth of pathogen was also increased. Both Eucalyptus (26.86%) and Lantana (26.15%) were on par with each other at 5% concentration followed by Garlic with inhibition of 24.97%. The least inhibition was observed in Pongamia leaf extract at all the three concentrations. [16] Who reported that *L. camara* presents secreting trichomes and idioblasts. These structures are known to secrete lipidic substances, alkaloids, sesquiterpene lactones and flavonoids which are responsible for antifungal activity. The results were supported with findings of [17] who reported that the maximum inhibition of growth of *P. oryzae* in Neem (41.53%) and Eucalyptus (42.80%) at 15% concentration. [18] Reported that higher concentration of neem extract was highly effective in checking the mycelial growth of *P. oryzae*. [19, 20]. Reported that neem leaf extracts reduced the mycelial growth of the fungus *P. oryzae*.

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