International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2017; 5(4): 506-509

Received: 25-05-2017
Accepted: 26-06-2017

Akhilesh Kumar Kulmitra
Department of Plant Pathology, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

VB Sanath Kumar
Department of Plant Pathology, University of Agricultural Sciences, GKVK, Bangalore, Karnataka, India

Thejesha AG
Department of Plant Pathology, University of Agricultural Sciences, GKVK, Bangalore, Karnataka, India

Amlan Ghosh
Department of Soil Science and Agricultural Chemistry, University of Agricultural Sciences, GKVK, Bangalore, Karnataka, India

Parmeshwar Sahu
Department of Genetics and Plant Breeding, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

Correspondence
Akhilesh Kumar Kulmitra
Department of Plant Pathology, Indira Gandhi Krishi Vishwavidyalaya, Raipur, Chhattisgarh, India

In vitro evaluation of fungicides against *Pyricularia oryzae* (Cav.) causing rice blast disease

Akhilesh Kumar Kulmitra, VB Sanath Kumar, Thejesha AG, Amlan Ghosh and Parmeshwar Sahu

Abstract
The present *In-vitro* study was conducted at pathology laboratory, University of agricultural sciences, GKVK, Bangalore, Karnataka, India during February 2016 to evaluate fungicides against rice blast disease caused by *Pyricularia oryzae* (Cav.). The six different fungicides *viz.,* Tebuconazole + Trifloxystrobin (50% + 25%) WG, Tebuconazole (25.9% EC), Azoxystrobin (23% EC), Captan (50% WP), Carbendazim (50% WP), and Tricyclazole (75% WP) were tested at three different concentrations of 50, 100 and 150ppm by using the poisoned food technique. Among the six different fungicides, highest per cent inhibition of mycelial growth of fungus was recorded in Tebuconazole + Trifloxystrobin (50% + 25%) WG (98.40, 99.90 and 99.90%) at all the concentration tested with mean of 99.40 per cent followed by Tebuconazole (25.9% EC) with the inhibition of 97.73, 99.90 and 99.90 per cent respectively with mean of 99.18 per cent. Minimum inhibition was observed in Tricyclazole (75% WP), with 55.83, 63.62 and 70.52 per cent inhibition at 50, 100 and 150ppm concentration respectively with a mean of 63.66 per cent. In general, the inhibition of radial growth of fungus increased with increase in concentration of each fungicide.

Keywords: fungicide, mycelial growth, percent inhibition, poisoned food technique, *Pyricularia oryzae*

Introduction
Rice, cereal grain (*Oryza sativa*) of the grass family (Graminae), probably native to the deltas of the great Asian rivers the Ganges, the Chang (Yangtze), and the Tigris and Euphrates. The plant is an annual, from 2 to 6 ft (61-183cm) tall, with a round, jointed stem, long, pointed leaves and edible seeds borne in a dense head on separate stalk.

Rice is Asia’s economically and culturally most important food crop and its production is regarded as the single most important economic activity on the planet. Rice is their major source of food of More than 2.7 billion peoples. Human consumption accounts for 85% of total production for rice, by the year 2025, this number will grow to 3.9 billion people. Over 90 percent of the world’s rice is produced and consumed in the Asia-Pacific Region. Rice is also the most important crop to millions of small farmers who grow it on millions of hectares throughout the region, and to the many landless workers who derive income from working on these farms. In the future, it is imperative that rice production continue to grow at least as rapidly as the population, if not faster.

Rice provides 21% of global human per capita energy and 15% of per capita protein. Although rice protein ranks high in nutritional quality among cereals, protein content is modest. Rice also provides minerals, vitamins, and fiber, although all constituents except carbohydrates are reduced by milling. Although the average yield per area did increase tremendously in the last forty years, the yield gap between economically optimal and actual yields remains large in many farmer’s fields and in many countries. This yield gap may be caused by unfavorable environmental conditions and limited material inputs, but inefficient production technologies and lacking knowledge contribute greatly.

The world’s estimated rice production is 496.0 million metric tons during 2016 (Anon, 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 (Anon, 2016) [1]. Rice is grown throughout India in all the states. The major rice growing states of India are West Bengal, Uttar Pradesh, Bihar, Madhya Pradesh, Orissa, Andhra Pradesh, Karnataka and Chhattisgarh.

Rice suffers from many diseases caused by fungi, bacteria, viruses, phytoplasma, nematodes and other non-parasitic disorders. Among the fungal diseases, blast is considered as a major threat to rice production because of its wide spread distribution and its destructiveness under favourable conditions. The Commonwealth Mycological Institute has recorded its presence from 85 countries throughout the world. Paddy blast is generally considered as the principal disease of rice and is caused by a fungus belonging to the Ascomycete *Pyricularia oryzae* Cavara (teleomorph= *Magnaporthe grisea* (Hebert) Barr Comb nov.). Losses due to the blast disease may range up to 90 per cent depending upon the component of the plant infected. *M. grisea* infects above ground parts of the plant, but neck blast and the panicle blast are the most damaging phases of the disease and have been shown to significantly reduce yield, grain weight and milling quality.

The pathogen may infect all the above ground parts of a rice plant at different growth stages *viz.*, leaf, collar, node, internodes, base or neck and other parts of the panicle and sometimes the leaf sheath. A typical blast lesion on a rice leaf is gray at the centre, has a dark border and it is spindle-shaped.

Materials and methods

The *In-vitro* study was conducted at pathology laboratory, University of agricultural sciences, GKVK, Bangalore, Karnataka, India during February 2016 to evaluate fungicides for their effect on the growth of fungus by using Poison food technique (Nene and Thapliyal, 1982) [12]. The different fungicides tested are listed in table 1.

Molten sterilized convenient media was prepared and autoclaved. The medium was cooled to 40 ^{0}C. The fungicides were dissolved in sterilized water to make stock solution. The stock solution of various fungicides was filter sterilized. Then appropriate quantity of stock solution was added to media, so as to get a required concentration and the flasks were agitated gently so as to disperse the fungicidal solution thoroughly into agar medium. About 15 to 20 ml of poisoned media was poured into 90mm Petri plates and allowed to solidify. The plates were rotated in clockwise direction to aid in uniform distribution of the medium. The actively growing peripheral growth of seven days old culture of fungus was carefully cut under aseptic condition by a cork borer and transferred to center of each Petri plates containing the poisoned medium. Suitable control was maintained in which the fungal pathogen was grown under similar conditions on media without poisoning the medium. Inoculated plates were incubated at 28±1 ^{0}C for fourteen days and the colony diameter was recorded after fourteen days of incubation by measuring the radial growth of the fungus in two directions at right angle to each other and average diameter was calculated. The per cent inhibition of growth over control was determined (Vincent, 1947) [14].

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

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

Statistical analysis

Analysis and interpretation of the experimental data was done by using completely randomized design (CRD) and Factorial CRD for laboratory studies ANOVA (Gomez and Gomez, 1984; Hosmand, 1988) [5, 8].

Table 1: List of fungicides used for *in vitro* evaluation against *Pyricularia oryzae*

Sl. No.	Common name	Trade name	Company name	Chemical name
Contact fungicides				
1	Captan	Captaf 50% WP	Makhteshim- Agan India Pvt. Ltd.,	N-(Trichloromethyl thio-4-cyclohexane-1,2, dicarboximide)
Combi-products				
1	Tebuconazole + Trifloxystrobin	Nativo (50% + 25%) WG	Bayer crop science AG. Germany.	1-(4-Chlorphenyl)-4,4-dimethyl-3-[1,2,4] triazol-1-ylmethyl-pentan-3-ol + (E,E)-methoxyimino-{2-{1-[3-trifluoromethyl-phenyl-ethyllideneaminooxymethyl]-phenyl} acetic acid methyl ester
Systemic fungicides				
1	Azoxystrobin	Amistar 23% EC	Syngenta India Ltd.,	Methyl (E)-2-[[6-(2-cyanophenoxy)-4-pyrimidinyl]oxy]-α-(methoxymethylene)benzeneacetate
2	Carbendazim	Bavistin 50% WP	Saraswati Agro Chemicals India Pvt. Ltd.,	Methyl 2 benzimidazole carbamate
3	Tebuconazole	Folicur 25.9% EC	Bayer crop science Ltd.,	α-[2-(4-chlorophenyl)ethyl]-α-(1,1- dimethyl)-1H-1,2,4-triazole-1-ethanol
4	Tricyclazole	Beam 75% WP	Dow crop science India Pvt. Ltd.,	5 – methyl – 1, 2, 4 – triazolo [3,4 – b] [1,3] benzothiazole

Results

Efficacy of four systemic, one contact and one combi product were evaluated at three different concentrations by following poison food technique. Per cent inhibition over control was worked out based on the test fungal growth in control plate. The results thus obtained have been presented in Table 2 and depicted in Fig. 1 and Plate 1.

Systemic fungicides

Inhibition of mycelial growth of *P. oryzae* was tested on different systemic fungicides *viz.*, carbendazim, tebuconozole, azoxystrobin, tricyclazole. Among fungicides tested tebuconozole and azoxystrobin were found to be highly effective at all three concentrations (50,100 and 150ppm) tested and these inhibited (99.40%) growth of *P. oryzae* and were significantly superior over control. Carbendazim (66.16%) and tricyclazole (63.66%) show moderately inhibition of the mycelial growth of *P. oryzae* at 50, 100 and 150ppm and it were comparable to control.

Contact fungicides and combi product

Efficacy of contact and combi product fungicides were tested against the pathogen and the data revealed that (Table 21, Fig. 5), contact fungicide, captan was effective at higher concentration as it inhibited the mycelial growth *P. oryzae* up to 67.66, 72.73 and 74.93 per cent at 50, 100 and 150ppm respectively. Whereas combi product, tebuconazole 50% + trifloxystrobin 25% (Nativo) showed 99.4 per cent inhibition at all the concentration (50,100 and 150ppm) tested.

Table 2: *In vitro* evaluation of fungicides against *Pyricularia oryzae*

Treatments	Fungicides	Mean per cent mycelial inhibition			
		Concentration (ppm)			
		50	100	150	Mean (%)
T1	Tebuconazole + Trifloxystrobin (50% + 25%) WG	98.40	99.90	99.90	99.40
T2	Tebuconazole (25.9% EC)	97.73	99.90	99.90	99.18
T3	Azoxystrobin (23% EC)	88.29	88.81	88.82	88.64
T4	Captan (50% WP)	67.66	72.73	74.93	71.77
T5	Carbendazim (50% WP)	63.29	65.42	69.76	66.16
T6	Tricyclazole (75% WP)	55.83	63.62	70.52	63.66
		Treatment	Concentrations	T X C	
	SEm±	0.31	0.22	0.54	
	C.D at 1%	1.21	0.85	2.09	
	CV %	1.16			

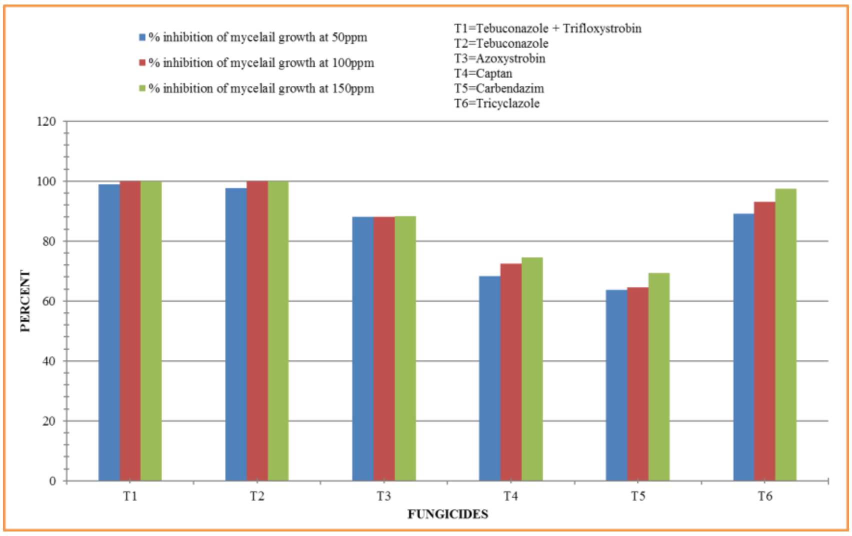

Fig 1: Effect of fungicides on the mycelial growth of *Pyricularia oryzae*

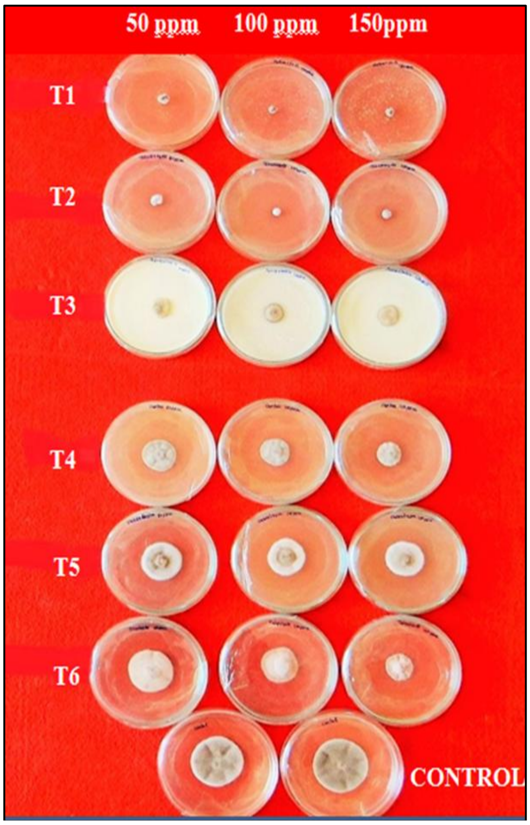

Plate 1: Effect of fungicides on the mycelial growth of *P. oryzae*.

Legend:

T1: Tebuconazole + Trifloxystrobin **T2:** Tebuconazole

T3: Azoxystrobin **T4:** Captan

T5: Carbendazim **T6:** Tricyclazole

Discussion

Efficacy of four systemic, one contact and one combi product were evaluated at three different concentrations by following poison food technique.

Systemic fungicides

Inhibition of mycelial growth of *P. oryzae* was tested on different systemic fungicides *viz.*, carbendazim, tebuconozole, azoxystrobin, tricyclazole. Among fungicides tested tebuconozole and azoxystrobin were found to be to be highly effective at all concentrations tested and these inhibited 99.4 per cent growth of *P. oryzae* and were significantly superior over control. Carbendazim and tricyclazole showed moderately inhibition of the mycelial growth of *P. oryzae* at 50, 100 and 150ppm and it was comparable to control.

Contact fungicides and combi product

Efficacy of contact fungicide captan and combi product (tebuconazole + trifloxystrobin) fungicides were tested against the pathogen and the data revealed that (Table 13 Fig. 10), contact fungicide, captan was effective at higher concentration as it inhibited the mycelial growth *P. oryzae* up to 67.66, 72.73 and 74.93 per cent at 50, 100 and 150ppm respectively. Whereas combi product, tebuconazole 50% + trifloxystrobin 25% (Nativo) showed 99.4 per cent inhibition at all the concentration tested.

The results of present investigation are in confirmatory with the findings of earlier workers. Viswanathan and Narayanasamy (1991) [15] and Gohel *et al*., (2008) [4] reported that tricyclazole was effective, Kapooria and Hairwadzi (1994) [10], Arun kumar and Singh (1995) [2], Anwar *et al*., (2002) [1], Hajano *et al.*, (2012) [6] and Jamal *et al*., (2012) [9] reported that carbendazim was effective, Haq *et al.*, (2002) [7], reported that captan, was effective, Mohan *et al.*, (2011) [11] reported that tebuconazole, tebuconozole + trifloxystrobin were effective, Bhojyanaik *et al.*, (2014) [3] reported that captan and tricyclazole were effective, Netam *et al.*, (2014) [13] reported that tricyclazole and carbendazim were effective against *P. oryzae* causing rice blast.

In the present study the effective fungicides *viz.*, tebuconazole, tebuconozole + trifloxystrobin, azoxystrobin and carbendazim probably may act as antifungal agents and imparts its poisoning effect on metabolic process of pathogen, therefore, the growth of the *P.oryzae* might be adversely affected.

Acknowledgement

The author wishes to thank Professor V. B. Sanath Kumar, University of Agricultural Sciences, Bangalore, Karnataka, India for his sustained interest in this work and the preparation of this paper. The award of ICAR –JRF (Junior research fellowship) to the author is also gratefully acknowledged.

References

1. Anonymous. Statistical database. 2016. http://www.fao.org.
2. Anwar A, Bhat GN, Singhara GN. Management of sheath blight and blast in rice through seed treatment. Ann. Pl. Prot. Sci. 2002; 10:285-287.
3. Arun K, Singh RA. Differential response of *Pyricularia grisea* isolates from rice, finger millet and pearl millet to media, temperature, pH and light. Ind. J. Mycol. and Plant pathol. 1995; 25: 238-242.
4. Bhojyanaik V, Jamadar MM. *In vitro* bioassay of different fungicides against blast of pearl millet caused by *Pyricularia grisea* (Cooke.) Sacc. Karnataka, J. Agric. Sci. 2014; 27(1):88-90.
5. Gohel NM, Chauhan HL, Mehta AN. Bio-efficacy of fungicides against *Pyricularia oryzae* the incitant of rice blast. J. Plant Dis. Sci. 2008; 3(2):189-192.
6. Gomez KA, Gomez AA. Statistical procedures for agricultural research with emphasis on rice. International rice research institute, los banos, philippines, 1984, 268.
7. Hajano J, Lodhi MA, Mumtaz AP, Khanzada MA, Shah SG. *In vitro* evaluation of fungicides, plant extracts and bio-control agents against rice blast pathogen *Magnaporthe oryzae* Couch. Pak. J. Bot. 2012; 44(5):775-1778.
8. Haq IM, Adnan MF, Jamil FF, Rehman A. Screening of rice germplasm against *Pyricularia oryzae* and evaluation of various fungitoxicants for control of disease. Pak. J. Pythopath. 2002; 14:32-35.
9. Hosmand RA. Statistical methods for agricultural sciences. timber press, portland, oregon, USA, 1988, 405.
10. Jamal-U-Ddin, Hajano Mubeen, Lodhi A, Mumtaz A, Pathan Ali et al. *In vitro* evaluation of fungicide, plant extract and bio-control agent against rice blast pathogen *Magnaporthe oryzae*., Pak. J. bot., 2012; 44(5):1775-1778.
11. Kapooria RG, Hairwadzi E. The effect of carbandazim on the growth of *P. oryzae,* Ghana J. Sci. 1994; 21:33-34.
12. Mohan C, Amrinder K, Sandeep R. *In vitro* evaluation of different fungicides against *Pyricularia grisea*. Pl. Dis. Res. 2011; 26(2):178.
13. Nene YL, Thaplial PN. Fungicides in plant disease control, Oxford and IBH Publishing House, New Delhi, 1982, 163.
14. Netam RS, Tiwari RKS, Bahadur AN, Shankar D. *In vitro* and *in vivo* efficacy of fungicides against *Pyricularia grisea* causing finger millet blast disease. Internat. J. Plant Protec. 2014; 7(1):137-142.
15. Vincent JM. Distribution of fungal hyphae in the presence of certain inhibitors. Nature, 1947; 159:850.
16. Viswanathan R, Narayanasamy P. Effect of tricyclazole on the physiology of *Pyricularia oryzae. Zeitschrift fuer Pflanzenkrankheiten* and *Pflanzenschutz* (Germany, F. R.). 1991; 98(2):205-212.