International Journal of Chemical Studies

P-ISSN: 2349–8528
E-ISSN: 2321–4902
IJCS 2019; 7(5): 1072-1076

Received: 04-07-2019
Accepted: 05-08-2019

Kavanashree K
Department of Plant Pathology, Centre for Plant Protection Studies, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Ramanathan A
Department of Plant Pathology, Centre for Plant Protection Studies, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Darshan K
Department of Plant Pathology, Centre for Plant Protection Studies, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

Correspondence
Kavanashree K
Department of Plant Pathology, Centre for Plant Protection Studies, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India

In vitro evaluation of single and combined formulations of the fungicides against rice blast caused by *Magnaporthe oryzae* Cav.

Kavanashree K, Ramanathan A and Darshan K

Abstract
Rice blast is one amongst the most devastating diseases caused by *Magnaporthe oryzae* Cav. (anamorph *Pyricularia oryzae* (Cooke) Sacc.) and are major constraints in the global rice production. Amongst the various strategies used for managing rice diseases, fungicides are of vital importance. So, in order to assess the efficacy, the seven commercial fungicides were tested under *in vitro* condition for their inhibitory activity through poisoned food technique and spore germination test at different concentration. *In vitro* evaluation of fungicides against *M. oryzae* disclosed that tricyclazole + tebuconazole (36% SC), tebuconazole 25% SC, hexaconazole 5% EC, zineb 68% + hexaconazole 4% WP and tebuconazole 50% + trifloxystrobin 25% WG completely inhibited the growth of fungus and germination of fungal spores in all concentration when compared with the control. Carbendazim 50% WP suppressed the blast pathogen in all concentrations except at 6.25% of recommended dose but it could inhibit the germination of spores only up to 50% of the recommended dose whereas tricyclazole 75% WP failed to inhibit completely the growth of fungal mycelium and spore germination.

Keywords: Rice, *Magnaporthe oryzae*, fungicides, poisoned food technique, tricyclazole

Introduction
Globally, rice (*Oryza sativa* L.) serves as primary source of food. It is an important and staple food for quite a half the world's population (Pooja and Katoch, 2014) [19]. The calorie intake from rice consumed by the world population is more than 23 per cent. India is said to be center of origin and diversity of rice and Asian countries are the major contributors in the production (Hayasaka *et al*., 2008; Kumar *et al.,* 2011) [8, 12]. India stands second in the world's rice production as 65% of its population depends on rice as a staple food. Rice production is affected by several biotic and abiotic constraints. Among the various biotic and abiotic constrainsts, rice blast disease is most disastrous globally and results 10-30 per cent loss during rice harvest (Skamnioti and Gurr, 2009; Xiao *et al.,* 2015) [23, 25]. Leaf blast, nodal blast, neck blast or panicle blast are the typical symptoms of this devastating disease. Neck blast causes highest yield loss since it affects the panicle directly (Ghatak *et al.,* 2013) [2]. In severe affected one the grain losses reach maximum level of 70 to 80 per cent (Padmanabhan, 1965; Hajano *et al*., 2011) [16, 5]. The disease favourable in areas with high rainfall and cooler climate. The first report of the disease was from Asia and now it is present in approximately 85 countries throughout the world. Soong Ying Shin reported the disease for the first time as 'rice fever disease' in his book 'Utilisation of Natural Sources' during 1637 in China (Manibushanrao, 1994) [13] and in Japan it was first reported as '*Imochi-byo'* in 1704 (Goto, 1955) [3]. It was reported as 'brusone' in Italy. The teleomorph of the organism is *Magnaporthe oryzae* Cav. (Ou, 1985) [15]. Sacc was named by Cavara in Italy (Cavara, 1891) [1] and subsequently in Japan (Shirai, 1896) [22]. In India, the disease was first recorded in Thanjavur delta of South India in 1918 by Mc Rae (1922). However, the disease gained attention during epidemics in 1919 (Padmanabhan, 1965) [16].
Cultural practices, use of resistant varieties, biological and chemical control are the different rice disease management strategies which control the disease to varied extent. Chemical control and use of resistant varieties are the most reliable control practices used worldwide (Prabhu *et al*., 2003) [20]. Moreover, the microorganism usually develops new biotypes leading to breakdown of resistance in the resistant varieties. Thus, use of chemical provides great opportunity for controlling rice diseases and focus on research has been shifted towards

developing new molecules that has high effectiveness. To date, fungicides are the most reliable strategy to achieve effective control of rice blast disease, especially where there is no sufficient genetic disease resistance (Groth, 2006; Morton and Staub, 2008) [4, 14]. Since this pathogen is highly variable, cultivars frequently become susceptible to this disease (Pooja and Katoch, 2014) [19]. The availability of new molecules to control this disease led to the present study. This study was carried out to evaluate the new molecules of single and combined formulations of the fungicides against rice blast.

Materials and Methods

Isolation and purification of rice blast pathogen

The blast pathogen infecting rice was isolated from the leaves of CO 39 variety collected from Paddy Breeding Station, Tamil Nadu Agricultural University, Coimbatore showing typical symptoms. The blast pathogen infected leaves were cut into small pieces of 1.0 to 1.5 cm, surface sterilized with 0.1 per cent mercuric chloride for thirty seconds and washed in sterile distilled water thrice. Then leaf bits were dried with sterilized filter paper. The sterilized leaf bits were placed on potato dextrose agar (PDA) plated Petri plates. The mycelial growth of the fungus was observed by incubating the plates at 26 ± 2 ºC for seven days. Further, the purification of the fungus was done by single spore isolation technique and were maintained on PDA slants for future studies (Ou, 1985) [15].

In vitro evaluation of fungicides

The fungicides with different doses were tested under *in vitro* condition for their inhibitory activity through poisoned food technique and inhibition of spore germination (Table 1).

Table 1: *In vitro* evaluation of different fungicides with different doses against rice blast pathogen

S. No	Chemicals	Dosage (g or ml/ l of water)					
		Recommended dose	75% of Recommended dose	50% of Recommended dose	25% of Recommended dose	12.5% of Recommended dose	6.25% of Recommended dose
1	Tricyclazole + tebuconazole (36%SC)	2.0ml	1.5ml	1.0ml	0.5ml	0.25ml	0.125ml
2	Tricyclazole + tebuconazole (36%SC)	2.25ml	1.6875ml	1.125ml	0.5625ml	0.28125ml	0.140625ml
3	Tricyclazole 75%WP	0.6g	0.45g	0.3g	0.15g	0.075g	0.0375g
4	Tebuconazole 25%SC	1.5ml	1.125ml	0.75ml	0.375ml	0.1875ml	0.09375ml
5	Hexaconazole 5%EC	2.0ml	1.5ml	1.0ml	0.5ml	0.25ml	0.125ml
6	Carbendazim 50%WP	1.0g	0.75g	0.5g	0.25g	0.125g	0.0625g
7	Zineb 68% + hexaconazole 4% WP	2.0g	1.5g	1.0g	0.5g	0.5g	0.5g
8	Tebuconazole 50% + trifloxystrobin 25% WG	1.0g	0.75g	0.5g	0.25g	0.25g	0.25g

Effect of fungicides on the mycelium growth of *M. oryzae*

The seven commercial fungicides tricyclazole + tebuconazole (36% SC), tricyclazole 75% WP, tebuconazole 25% SC, hexaconazole 5% EC, carbendazim 50% WP, zineb 68% + hexaconazole 4% WP and tebuconazole 50% + trifloxystrobin 25% WG were evaluated under *in vitro* against *M. oryzae* through poisoned food technique in the above-mentioned dosage. Fungicides were added to the 100ml sterilized PDA medium just before pouring. PDA medium without any fungicides served as control. A pinch of streptomycin sulphate was added to sterilized medium before pouring into Petri dishes to avoid bacterial contamination. Each treatment was replicated thrice. After solidification of the medium, nine-millimeter disc of pure culture of *M. oryzae* was placed in the center of Petri dishes and incubated. Radial mycelia growth of the test fungus was recorded in mm at 24 hours interval until the upper surface in control Petridish was fully covered with the mycelium of the fungus (Hajano *et al.*, 2012) [6].

$$\text{Per cent inhibition} = \frac{(C-T)}{C} \times 100$$

Where,

C = Radial growth of mycelium in fungicide unamended medium (control)

T = Radial growth of mycelium in fungicide amended medium

Effect of fungicides on sporulation and spore germination of *M. oryzae*

M. oryzae was grown on PDA for 15 days. Fungal discs (9mm) plugged out from the fungal grown media were aseptically transferred to glass slides (8 discs/slide), kept in sterile Petri dishes with moist cotton. Each petri dish was placed with two glass slides. The mycelial discs were observed under microscope for the presence of spores after 48 hours of incubation.

Seven fungicides were evaluated for its inhibitory activity against *M. oryzae* under *in vitro* condition. The spore germination inhibition studies were conducted by following the method described by Peterson (1941) [18]. Spore suspension of *M. oryzae* was prepared from the incubated pathogen discs as mentioned previously. Required concentration of test chemical was prepared by using sterile distilled water and filtered. Twenty micro liters of the solution was placed at the centre of a clean and sterilized cavity slide and allowed it to dry at room temperature (30-35 ^{0}C). Twenty micro liter of spore suspension was prepared in sterile water and placed on the same spot where fungicidal suspension was placed. Later slides were placed in petri plates with moisted cotton. The moist chambers with slides in it were incubated. Spore suspension without fungicides acted as control. Observations on number of spores germinated were recorded 24 h after incubation under high power (40X objective) image analyser. Three replications were maintained for each chemical. From each replication five microscopic fields were observed (Hegde, 1998) [9]. Per cent inhibition was calculated by using the following formula (Verma and Singh, 1987) [24].

$$\text{Per cent inhibition} = \frac{(C-T)}{C} \times 100$$

Where,

C = number of spores germinated in control

T = number of spores germinated in treatment

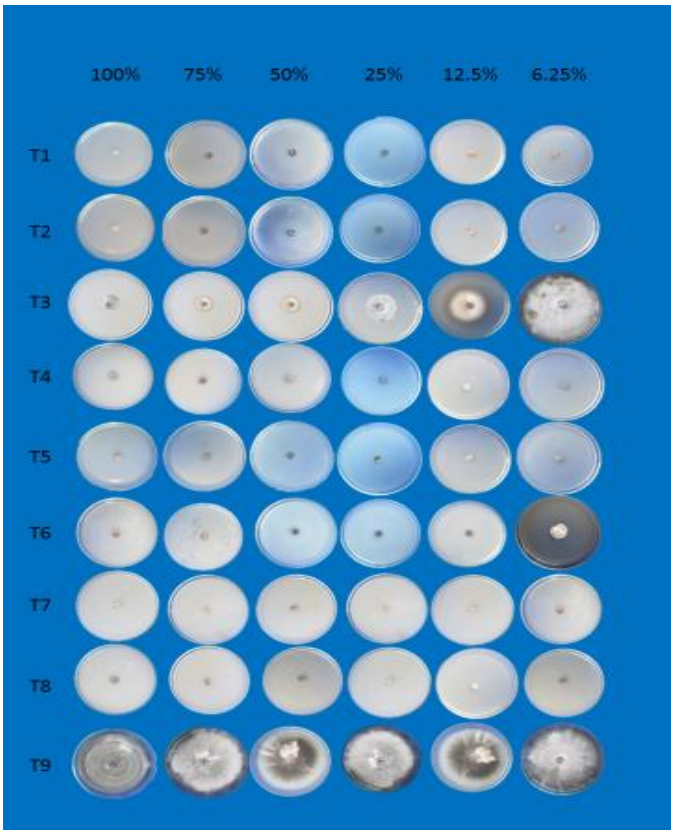
Results and Discussion

In vitro evaluation of fungicides on mycelial growth of blast pathogen

Effect of seven different fungicides was studied against rice blast pathogen through poison food technique (Table 2, Figure 1). Mycelial growth of blast pathogen was nil in all 6 concentrations of the 5 fungicides tricyclazole + tebuconazole (36% SC), tebuconazole 25% SC, hexaconazole 5% EC, zineb 68% + hexaconazole 4% WP and tebuconazole 50% + trifloxystrobin 25% WG. Growth was low at initial concentration and was full at 6.25% of the recommended dose of tricyclazole 75% WP. Carbendazim 50% WP suppressed the blast pathogen in all concentrations except at 6.25% of recommended dose. The results are in accordance with Hajano *et al*. (2012) [6] who tested five fungicides viz., thiophanate-methyl, carbendazim, fosetyl-aluminum, mancozeb and copper oxychloride against *M. oryzae* under *in vitro* condition with 3 different concentrations of 100, 1000 and 10000 ppm. Amongst five fungicides, mancozeb was found highly effective, which completely suppressed the fungal mycelial growth at 1000 and 10,000ppm. All other fungicides failed to inhibit the mycelial growth of the fungus completely. Mycelial growth of the test fungus at 10,000ppm of thiophanate-methyl, carbendazim, fosetyl aluminium and copper oxychloride were 20.84 mm, 20.66mm, 12.80 mm and 22.16 mm respectively. The similar results were also reported by Kulmitra *et al*. (2017) [11] during *in vitro* evaluation of fungicides against blast pathogen. Among the tested six chemicals, tebuconazole + trifloxystrobin (50% + 25%) WG inhibited the pathogen effectively (98.40, 99.90 and 99.90%) with the mean of 99.40 at 50, 100 and 150ppm concentration followed by tebuconazole (25.9% EC) (97.73, 99.90 and 99.90) with mean inhibition 99.18 per cent. Minimum inhibition was recorded in tricyclazole (75% WP) with a mean of 63.66 per cent.

Effect of fungicides on percent inhibition of *M. oryzae*

Effect of seven different fungicides was studied against rice blast pathogen through poisoned food technique. Inhibition percent was assessed (Table 2, Figure 1). Fungicides tricyclazole + tebuconazole (36% SC), tebuconazole 25% SC, hexaconazole 5% EC, zineb 68% + hexaconazole 4% WP and tebuconazole 50% + trifloxystrobin 25% WG inhibited the blast fungus completely in all concentration (100%). Carbendazim 50%WP inhibited the pathogen upto 100% in all concentration except in 6.25% of recommended dose whereas tricyclazole 75% WP was least effective and did not inhibit the fungus completely even at higher concentration. Similar result was showed by Haq *et al*. (2002) [7] in the experiment of evaluation of various fungicides like Captan, Acrobat, Bayeltan, Sunlet, Dithane M-45, Trimiltox and Derosal in controlling the macelial growth of *Pyricularia oryzae* under the laboratory conditions. The results depicted the effectiveness of Captan and Acrobat.



T1- Tricyclazole + tebuconazole (36% SC)
T2- Tricyclazole + tebuconazole (36% SC)
T3- Tricyclazole 75% WP
T4- Tebuconazole 25% SC
T5- Hexaconazole 5% EC
T6- Zineb 68% + hexaconazole 4% WP
T7- Zineb 68% + hexaconazole 4% WP
T8- Tebuconazole 50% + trifloxystrobin 25% WG
T9- Control

Fig 1: Efficacy of fungicides against blast pathogen

Table 2: Effect of different fungicides against percent inhinition of blast pathogen through Poison Food Technique (PFT)

SI No.	Treatments	Recommended dose (g or ml/l of water)	Percent inhibition (%) *					
			Recommended dose	75% of Recommended dose	50% of Recommended dose	25% of recommended dose	12.5% of Recommended dose	6.25% of Recommended dose
1.	Tricyclazole + Tebuconazole (36%SC)	2.0ml	100.00^{a} (89.45)	100.00^{a} (89.45)	100.00^{a} (89.45)	100.00^{a} (89.45)	100.00^{a} (89.45)	100.00^{a} (89.45)
2.	Tricyclazole + Tebuconazole (36%SC)	2.25ml	100.00^{a} (89.45)	100.00^{a} (89.45)	100.00^{a} (89.45)	100.00^{a} (89.45)	100.00^{a} (89.45)	100.00^{a} (89.45)
3.	Tricyclazole 75%WP	0.6g	84.07^{b} (66.49)	79.63^{b} (63.18)	73.33^{b} (58.91)	59.26^{b} (50.34)	49.67^{b} (4.81)	0.00^{c} (0.55)
4.	Tebuconazole 25%WG	1.5ml	100.00^{a} (89.45)	100.00^{a} (89.45)	100.00^{a} (89.45)	100.00^{a} (89.45)	100.00^{a} (89.45)	100.00^{a} (89.45)
5.	Hexaconazole 5%EC	2.0ml	100.00^{a} (89.45)	100.00^{a} (89.45)	100.00^{a} (89.45)	100.00^{a} (89.45)	100.00^{a} (89.45)	100.00^{a} (89.45)
6.	Carbendazim 50%WP	1.0g	100.00^{a} (89.45)	100.00^{a} (89.45)	100.00^{a} (89.45)	100.00^{a} (89.45)	100.00^{a} (89.45)	84.07^{b} (66.49)
7.	Zineb 68% + Hexaconazole 4% WP	2.0g	100.00^{a} (89.45)	100.00^{a} (89.45)	100.00^{a} (89.45)	100.00^{a} (89.45)	100.00^{a} (89.45)	100.00^{a} (89.45)
8.	Tebuconazole 50% +	1.0g	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}	100.00^{a}

	Trifloxystrobin 25% WG		(89.45)	(89.45)	(89.45)	(89.45)	(89.45)	(89.45)
9.	Control		0.00^{c} (0.55)	0.00^{c} (0.55)	0.00^{c} (0.55)	0.00^{c} (0.55)	0.00^{c} (0.55)	0.00^{c} (0.55)
	CD(.05)		0.80	0.53	0.41	0.43	0.50	0.28
	SEd		0.28	0.25	0.20	0.20	0.24	0.13

* Values are mean of three replications, Figures in parentheses represent arc sine transformation

Spore germination assay against *M. oryzae*

Seven fungicides tested for their efficacy against germination of spores of *M. oryzae*. All the fungicides tested gave cent per cent inhibition at recommended concentration except tricyclazole 75% WP. Highest percent inhibition over control was observed in tricyclazole + tebuconazole (36% SC), tebuconazole 25% SC, hexaconazole 5% EC, zineb 68% + hexaconazole 4% WP and tebuconazole 50% + trifloxystrobin 25% WG even at 6.25% of recommended dose. Carbendazim 50% WP was found ineffective below 50% of the recommended dose whereas tricyclazole 75% WP was ineffective at all the concentrations and spores were germinated (Table 3, Figure 2). The findings are in accordance with the results of studies conducted by Raj *et al*. (2017) [21] who reported that tricyclazole proved to be the most effective followed by propiconazole whereas mancozeb was found as the least effective fungicide. The complete spore germination inhibition was observed at 50 ppm concentration of tricyclazole, propiconazole, azoxystrobin + difenoconazole, trifloxystrobin + tebuconazole and at 100 ppm for difenoconazole and mancozeb.

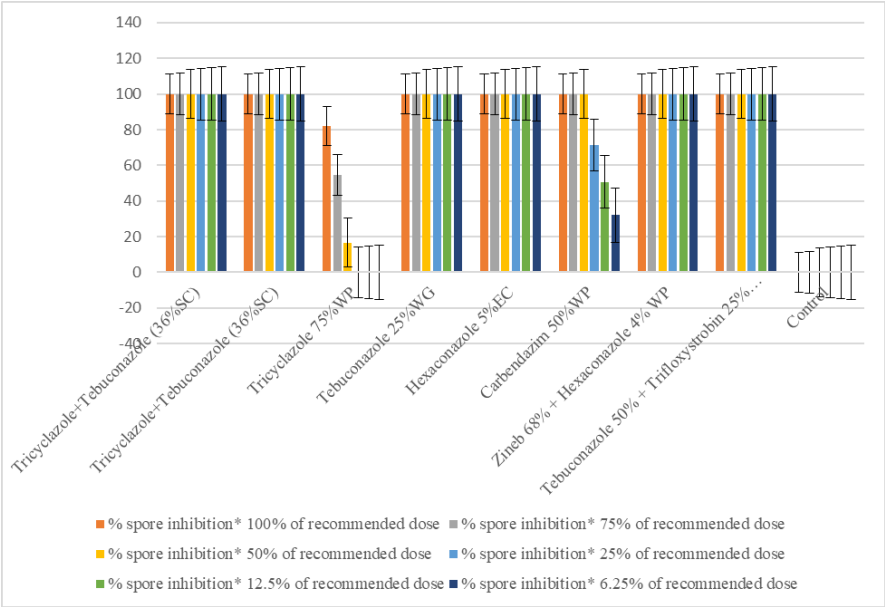


Fig 2: Inhibitory effect of fungicides on blast spore germination

Table 3: Inhibitory effect of fungicides on blast spore germination under *in vitro* condition

SI No.	Treatments	Recommended Dose (g or ml/l of water)	% spore germination*					
			Recommended dose	75% of Recommended dose	50% of Recommended dose	25% of Recommended dose	12.5% of Recommended dose	6.25% of Recommended dose
1.	Tricyclazole + Tebuconazole (36%SC)	2.0ml	0.00^{a} (0.55)	0.00^{a} (0.55)	0.00^{a} (0.55)	0.00^{a} (0.55)	0.00^{a} (0.55)	0.00^{a} (0.55)
2.	Tricyclazole + Tebuconazole (36%SC)	2.25ml	0.00^{a} (0.55)	0.00^{a} (0.55)	0.00^{a} (0.55)	0.00^{a} (0.55)	0.00^{a} (0.55)	0.00^{a} (0.55)
3.	Tricyclazole 75%WP	0.6g	17.97^{b} (25.08)	45.45^{b} (42.39)	83.33^{b} (65.91)	100.00^{c} (89.45)	100.00^{c} (89.45)	100.00^{c} (89.45)
4.	Tebuconazole 25%WG	1.5ml	0.00^{a} (0.55)	0.00^{a} (0.55)	0.00^{a} (0.55)	0.00^{a} (0.55)	0.00^{a} (0.55)	0.00^{a} (0.55)
5.	Hexaconazole 5%EC	2.0ml	0.00^{a} (0.55)	0.00^{a} (0.55)	0.00^{a} (0.55)	0.00^{a} (0.55)	0.00^{a} (0.55)	0.00^{a} (0.55)
6.	Carbendazim 50%WP	1.0g	0.00^{a} (0.55)	0.00^{a} (0.55)	0.00^{a} (0.55)	28.57^{b} (32.31)	49.30^{b} (44.60)	67.93^{b} (55.51)
7.	Zineb 68% + Hexaconazole 4% WP	2.0g	0.00^{a} (0.55)	0.00^{a} (0.55)	0.00^{a} (0.55)	0.00^{a} (0.55)	0.00^{a} (0.55)	0.00^{a} (0.55)
8.	Tebuconazole 50% + Trifloxystrobin 25% WG	1.0g	0.00^{a} (0.55)	0.00^{a} (0.55)	0.00^{a} (0.55)	0.00^{a} (0.55)	0.00^{a} (0.55)	0.00^{a} (0.55)
9.	Control		100.00^{c} (89.45)	100.00^{c} (89.45)	100.00^{c} (89.45)	100.00^{c} (89.45)	100.00^{c} (89.45)	100.00^{c} (89.45)
	CD(.05)		0.27	0.21	0.28	0.23	0.20	0.22
	SE.d		0.13	0.10	0.13	0.11	0.10	0.10

* Values are mean of three replications
Figures in parentheses represent arc sine transformation

Conclusion

Rice (*Oryza sativa* L.) is one of the vital staple food crops of the Asian countries and its productivity was affected by many fungal pathogens. Among this, blast caused by *M. oryzae* is a major disease of rice causing yield loss and serious threat to rice growers. It is a hemibiotrophic fungal pathogen as it uses the infection strategy that involves initial multiplication inside living host cells before switching to a devastating necrotrophic mode (Howard *et al.*, 1991; Park *et al.*, 2009) [10, 17]. *In vitro* evaluation of fungicides against *M. oryzae* revealed that tricyclazole + tebuconazole (36% SC), tebuconazole 25% SC, hexaconazole 5% EC, zineb 68% + hexaconazole 4% WP and tebuconazole 50% + trifloxystrobin 25% WG inhibited completely the growth of fungus and germination of fungal spores in all concentration. Carbendazim 50% WP suppressed the blast pathogen in all concentrations except at 6.25% of recommended dose but it could inhibit the germination of spores only upto 50% of the recommended dose whereas tricyclazole 75% WP failed to inhibit completely the growth of fungal mycelium and spore germination. In order to combat against this pathogen, the identification of effectiveness of new molecules and its integration in IDM is one of the tools to control rice blast disease effectively.

Acknowledgements

The authors would like to thank Professor & Head, Department of Plant Pathology and The Dean, School of Post Graduate Studies, Tamil Nadu Agricultural University, Coimbatore for providing facilities to successfully carry out the research program.

References

1. Cavara F. Fungi *Longobardiae exsiccati* sive mycetum specimina in *Longobardia collecta*, exsiccata et speciebus novis vel criticis, iconibus illustrata. Pugillus I. 1891; 18.
2. Ghatak A, Willocquet L, Savary S, Kumar J. Variability in aggressiveness of rice blast (*Magnaporthe oryzae*) isolates originating from rice leaves and necks: a case of pathogen specialization. PloS one. 2013; 8:66180.
3. Goto K. Histroy of the blast disease and changes in methods of control. Agricultural Improvement Bureau, Ministry of Agriculture and Forestry, Japan. 1955; 5:1-2.
4. Groth DE. Azoxystrobin rate and timing effects on rice head blast incidence and rice grain and milling yields. Plant Dis. 2006; 90:1055-1058.
5. Hajano JU, Pathan MA, Rajput QA, Lodhi MA. Rice blast microflora, symptomatology and pathogenicity. Int. J Agric. Sci. Vet. Med. 2011; 5:53-63.
6. Hajano J, Lodhi AM, Pathan MA, Khanzada MA, Shah GS. *In vitro* evaluation of fungicides, plant extracts and bio-control agents against rice blast pathogen *Magnaporthe oryzae* couch. Pakistan J Bot. 2012; 44(5):1775-1778.
7. Haq IM, Adnan MF, Jmil FF, Rehman A. Screening of rice germplasm against *Pyricularia oryzae* and evaluation of various fungitoxicants for control of disease. Pakistan J Phytopathol. 2002; 14:32-35.
8. Hayasaka T, Fujii H, Ishiguro K. The role of silicon in preventing appressorial penetration by the rice blast fungus. Phytopathol. 2008; 98:1038-1044.
9. Hegde YR. Biology and Management of False Smut of Rice (*Oryza sativa* L.) caused by *Claviceps oryzae-sativa* Hashioka in Karnataka. University of Agricultural Sciences. 1988.
10. Howard RJ, Ferrari MA, Roach DH, Money NP. Penetration of hard substrates by a fungus employing enormous turgor pressures. Proceedings of the National Academy of Sciences. 1991; 8:11281-11284.
11. Kulmitra AK, Sanath Kumar VB, Thejesha AG, Ghosh A, Sahu P. *In vitro* evaluation of fungicides against *Pyricularia oryzae* (Cav.) causing rice blast disease. Int. J Chem. Stud. 2017; 5(4):506-509.
12. Kumar KVK, Reddy MS, Kloepper JW, Lawrence KS, Zhou XG, Groth DE, *et al*. Commercial potential of microbial inoculants for sheath blight management and yield enhancement of rice. In: Bacteria in Agrobiology: Crop Ecosystem, Maheshwari, D. K. (Ed). 2011; 237-264. DOI: 10.1007/978-3-642-18357-7-9.
13. Manibhushanrao K. Rice blast disease. Daya Publishing House, Delhi. 1994; p 1.
14. Morton V, Staub T. A Short History of Fungicides. Online, APS net Features. 2008; DOI: 10.1094/APSnetFeature-2008-0308
15. Ou SH. Rice Diseases. International Rice Research Institute, Los Banos, Laguna, Philippines. 1985; p. 11-12.
16. Padmanabhan SY. Breeding for blast resistance in India. In 'The rice blast disease', Ed. Johns Hopkins Press, Baltimore and Maryland, USA. 1965; p. 203-221.
17. Park JY, Jin J, Lee YW, Kang S, Lee YH. Rice blast fungus (*Magnaporthe grisea)* infects Arabidopsis via a mechanism distinct from that required for the infection of rice. Plant Physiol. 2009; 149:474-486.
18. Peterson P. The spore-germination method of evaluating fungicides. Phytopathology. 1941; 31:1108-1116.
19. Pooja K, Katoch A. Past, present and future of rice blast management. Plant Sci. Today. 2014; 1:165-173.
20. Prabhu AS, Filippi MC, Zimmermann FJP. Cultivar response to fungicide application in relation to rice blast control, productivity and sustainability. Pesquisa Agropecuária Brasileira. 2003; 38:11-17.
21. Raj R, Pannu PPS. Management of rice blast with different fungicides and potassium silicate under *in vitro* and *in vivo* condition. J. Plant Pathol. 2017; 99(3):707-712.
22. Shirai M. Notes on plants collected in Suruga, Totomi, Yamato and Kii. Botanical Magazine, Tokyo. 1896; 10:111-114.
23. Skamnioti P, Gurr SJ. Against the grain: safeguarding rice from rice blast disease. Trends in Biotechnology. 2009; 27(3):141-150.
24. Verma RK, Singh RA. Evaluation of fungicides and antibiotics against *Claviceps oryzae-sativae,* the incitant of false smut of rice. Pesticides. 1987; 21(10):40-42.
25. Xiao W, Yang Q, Sun D, Wung H, Guo T, Liu Y, *et al*. Identification of three major *R* genes responsible for broad-spectrum blast resistance in an *indica* rice accession. Mol. Breeding. 2015; 35:49.