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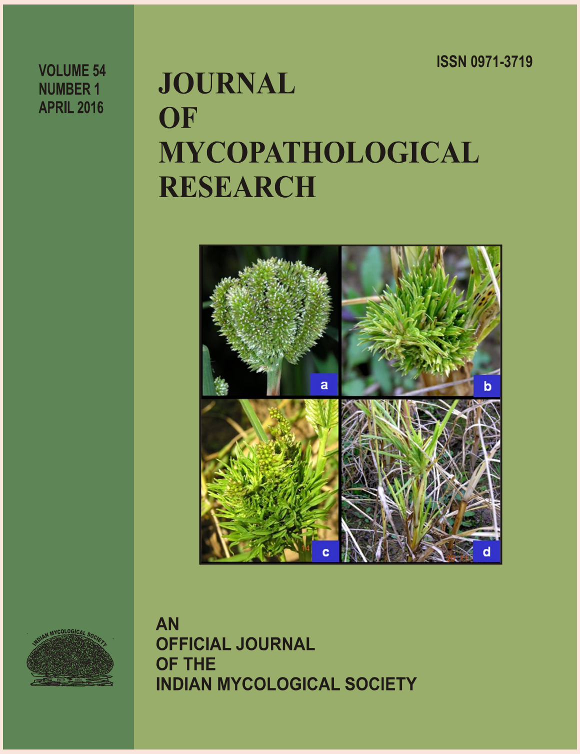

J. Mycopathol, Res, 54(1) : 71-75, 2016;
ISSN 0971-3719

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In vitro evaluation of some new fungicides against Blast and Sheath blight pathogens in rice

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Received : 12.06.2015 RMS Accepted : 17.07.2015 Published : 25.04.2016

Ten fungicides were evaluated in vitro for their potential against *Pyricularia oryzae* and *Rhizoctonia solani* causing blast and sheath blight in rice. Against P. oryzae, Tricyclazole 75WP and Azoxystrobin 18.2%+Difenoconazole 11.4% SC were found most effective resulting 84.31% and 80.00% reduction of mycelia growth at 100 ppm, respectively. Difenoconazole 25 EC was found effective at higher concentration (150 ppm) resulting 84.31% reduction of pathogen growth while Pencycuron 22.9SC was least effective against *P. oryzae*. EC50, an indicator of fungicide power against a particular fungus showed that Azoxystrobin 18.2%+ Difenoconazole 11.4% SC had highest toxicity against *P. oryzae* followed by Tricyclazole.75WP. Cyproconazole 50S and Azoxystrobin 18.2% + Difenoconazole 11.4%SC were found to be the most effective fungicides against *R. solani* resulting 100% reduction of pathogen growth at 150 ppm but Propiconazole 25EC was most toxic in term of its lowest EC50.

Key words: Fungicides, *Pyricularia oryzae*, *Rhizoctonia solani*, EC50

INTRODUCTION

Among the various fungal diseases of rice blast and sheath blight hold major attention during past few decades due to their recurrent appearance in all rice growing regions of the world. Rice blast caused by *Pyricularia oryzae*, an anamorph of *Magnaporthe grisea* attacks on stem nodes, leaves and all portions of the panicle and grains of rice (Seebold *et al,* 2004). Blast epidemic causes the complete defeat of seedling at the nursery and in field condition and accomplish 50-90% of total yield fatalities (Agrios, 2005). Sheath blight caused by *Rhizoctonia solani*, an anamorph of *Thanatephorus cucumeris* is another major production constraint in many rice growing areas especially in coastal and high humid regions. Loss in yield may upto 53% depending on the cultivar, environmental condition, stages at which the plants are infected and level of infection has been reported (Rajan, 1987; Roy, 1993). The disease is particularly important in intensive rice production systems (Savary and Mew, 1996). The natural infection of the sheath blight disease occurs at the seedling, tillering and booting stages of rice. The entire plant often gets killed under severe cases. The pathogen has a wide host range and can infect plants belonging to more than 32 plant families and 188 genera (Gangopadyay and Chakrabarti, 1982).

The most usual approaches for the management of the rice fungal diseases include planting of resistant cultivars, application of fungicides, manipulation of planting times, fertilizers and irrigations (Georgopoulos and Ziogas, 1992; Moletti, 1988; Mbodi *et al*, 1987). The inherent level of resistance in rice to blast and sheath blight is very low. For farmers in developing countries, like Vietnam, India, Indonesia planting resistant varieties is the easiest and safest method but the resistance of most varieties is readily overcome within short period of time (Bonman *et al*, 1992). Chemical control of the diseases, therefore, is the most widely accepted strategies to keep the severity below economic threshold level but due to sudden mutation in certain population the resistance to fungicides may developed and the virulent population causes infection even on resistant varieties. Approximately 30 fungicides belonging to benzimidazoles, triazoles, succinate dehydrogenase inhibitors, melanin biosynthesis inhibitors, strobulirins, antifungal antibiotics, etc. have been tested and registered in India for control of blast and sheath blight (Kumar *et al*, 2014) and several new molecules are under testing. Yet the efficiency of particular fungicides could vary from place to place or from dosage to dosage. Farmers are advised to revolve the fungi-toxicants used to prevent the infectious fungus form rising resistance against those fungicides (Tangdiabang and Pakki, 2006). Hence, information about efficient fungicides with different modes of action should be offered to farmers. In this view, the present investigation was undertaken to assess the efficacy of some new commercially available fungicides against blast and sheath blight diseases.

MATERIALS AND METHODS

The test pathogens *P. oryzae* and *R. solani* were isolated from the infected rice leaf and sheath collected from Instructional Farm, Uttar Banga Krishi Viswavidyalaya, Pundibari, Cooch Behar, West Bengal. The pure cultures of the pathogens were maintained on Rice Extract Agar (REA) and Potato Dextrose Agar slants, respectively at 4±1^{0}C.

The comparative toxicity of ten fungicides on the growth of the pathogens under in vitro condition was evaluated by poisoned food technique (Dhingra and Sinclair, 1985). Fungicides like Propiconazole 25EC (Tilt), Tricyclazole 75WP (Baan), Tricyclazole 34.2% + Propiconazole 10.7% (Filia 52.5 SE), Difenoconazole 25EC (Score), Cyproconazole 50S (Alto), Triamaphos 48EC (Kitazin), Carbendazim 50WP (Bavistin), Pencycuron 22.9SC (Monceren), Azoxystrobin 23SC (Amister) and Azoxystrobin 18.2%+Difenoconazole 11.4% SC (Amister Top 325 SC) at different concentrations (25, 50, 100 and 150 ppm) were used for in vitro assay. The fungicides were incorporated into the sterilized REA and PDA medium for the study on *P. oryzae* and *R. solani*, respectively. The sterilized petriplates containing amended media were inoculated with 6 mm disc of freshly prepared culture of the pathogens and incubated at 27±1^{0}C for 7 days. Five replications for each concentration of an individual fungicide along with check by inoculating mycelia disc on non-amended media were maintained. The efficacy of°the fungicides was expressed as percent of radial growth over control, which was calculated by using the formula I=(C-T/T)×100 Where, I= Per cent inhibition over control C= Radial growth in control and T= Radial growth in treatments. The EC50 (i.e., the effective concentration for 50% inhibition in growth) of each fungicides against each pathogens was also calculated by working out the linear equation from the calculated per cent inhibition.

RESULTS AND DISCUSSION

Results on *in vitro* evaluation of the fungicides against *P. oryzae* have been presented in Table 1. It was observed that with increasing concentration of fungicides the mycelia growth of P. oryzae reduced accordingly. Maximum inhibition of 70.59-84.31% had been recorded against Tricyclazole 75WP at 25 ppm-100 ppm. The result was statistically at par with Azoxystrobin 18.2% + Difenoconazole 11.4%SC (69.02, 75.69 and 80.00% reduction at 25, 50 and 100 ppm, respectively). However, at 150 ppm Tricyclazole 75WP significantly outperformed (88.63% reduction in growth over control) Azoxystrobin 18.2% + Difenoconazole 11.4%SC (81.57% reduction in growth over control). Although at lower concentrations (25-50 ppm) Difenoconazole 25EC was moderately effective but at higher concentration it was good enough to reduce the mycelia growth of *P. oryzae* (77.65 and 84.31% reduction at 100 and 150 ppm, respectively). Pencycuron 22.9SC was the least effective showing 18.82% reduction in growth at 150 ppm concentration. Lowest EC50 was calculated for Azoxystrobin 18.2% + Difenoconazole 11.4%SC (1.50 ppm) closely followed by Tricyclazole 75WP (2.93 ppm). The other

Table 1 : *In vitro* evaluation of fungicides against *P. oryzae*

Fungicide	Per cent reduction in growth over control				Linear equation	R^2	EC50 (ppm)
	25 ppm	50 ppm	100 ppm	150 ppm			
Propiconazole 25%EC	41.96	43.14	47.45	56.86	y=17.58x+15.37	0.792	93.29
Tricyclazole 75WP	70.59	78.82	84.31	88.63	y=22.63x+39.42	0.993	2.93
Tricyclazole 34.2% + Propiconazole10.7%	51.37	60.78	67.84	74.12	y=28.44x+11.81	0.995	22.02
Difenoconazole 25%EC	49.80	66.27	77.65	84.31	y=43.88x-10.27	0.990	23.63
Cyproconazole 50%S	52.55	59.61	71.37	79.22	y=34.43x+3.07	0.982	23.07
Triamaphos 48%EC	25.10	38.43	49.02	53.73	y=36.93x-25.59	0.991	111.39
Carbendazim 50%WP	32.16	53.33	64.31	71.37	y=49.29x-34.34	0.973	51.42
Pencycuron 12.5%SC	0.78	6.67	12.55	18.82	y=22.42x-31.06	0.982	4125.93
Azoxystrobin 23SC	53.33	59.22	61.57	69.41	y=18.60x+27.05	0.916	17.13
Azoxystrobin 18.2%+Difenoconazole 11.4% SC	69.02	75.69	80.00	81.57	y=16.16x+47.16	0.973	1.50
SEm±	3.37	1.72	1.58	1.12			
CD(P=0.05)	10.08	5.14	4.73	3.34			

test fungicides were in the rank of Azoxystrobin 23SC <Tricyclazole 34.2% + Propiconazole 10.7%SE < Cyproconazole 50S < Difenoconazole 25EC < Carbendazim 50WP< Propiconazole 25EC < Triamaphos 48EC whereas, Pencycuron 22.9SC showed highest EC50 (4125.93 ppm). Gohel *et al*, (2008) evaluated 19 fungicides against *M. oryzae* and found that Tricyclazole, Mancozeb, Carbendazim, Iprobenfos, Propiconazole and Edifenphos were highly effective against the test fungus. The results also corroborate earlier findings by Sood and Kapoor (1997), Ghaznafar *et al*, (2009), Jamal Uddin *et al.* (2011), Verma and Santhakumari (2012) and Singh *et al*, (2014).

The fungitoxicity of the test chemicals on R. solani revealed that as a general phenomenon with fungicide gradient the mycelial growth decreased significantly irrespective of fungicides concerned. Cyproconazole 50S and Azoxystrobin 18.2% + Difenoconazole 11.4%SC were found to be the most effective fungicides reducing the mycelia growth by 76.08-78.04% at 25 ppm to 100% at 150 ppm concentration. At 25, 50 and 100 ppm concentration, Propiconazole 25EC was statistically at par with Cyproconazole 50%S and Azoxystrobin 18.2% + Difenoconazole 11.4%SC exhibiting 73.73, 78.04 and 86.27% reduction in growth over control, respectively. Propiconazole 25EC at 150 ppm concentration resulted 90.98% reduction in mycelia growth of *R. solani* which statistically differed from the ability of Cyproconazole 50S and Azoxystrobin 18.2% + Difenoconazole 11.4%SC. Most of the other fungicides (Difenoconazole 25EC, Triamaphos 48EC, Carbendazim 50WP, Pencycuron 22.9SC and Azoxystrobin 25SC) at 100-150 ppm concentration resulted >50% growth inhibition. At lower concentration of 25ppm, 46.27% growth inhibition of *R. solani* was recorded with Carbendazim 50WP and was statistically at par with Triamaphos 48EC, Pencycuron 22.9SC and Azoxystrobin 23SC. Among all the fungicides, Tricyclazole 75WP showed least inhibition at all the concentrations evaluated. It was observed that Propiconazole 25EC had lowest EC50 (2.45 ppm) followed by Cyproconazole 50S and Azoxystrobin

Table 2 : *In vitro* evaluation of fungicides against *R. solani*

Fungicide	Per cent reduction in growth over control				Linear equation	R^2	EC50 (ppm)
	25 ppm	50 ppm	100 ppm	150 ppm			
Propiconazole 25%EC	73.73	78.04	86.27	90.98	y=22.56x+41.23	0.980	2.45
Tricyclazole 75WP	7.84	34.12	47.06	52.55	y=56.79x-67.87	0.953	119.00
Tricyclazole 34.2% + Propiconazole10.7%	54.51	72.94	77.25	80.00	y=31.41x+14.05	0.877	13.95
Difenoconazole 25%EC	59.61	69.41	75.69	79.61	y=25.31x+25.05	0.987	9.68
Cyproconazole 50%S	78.04	80.78	95.29	100.00	y=30.30x+33.42	0.930	3.53
Triamaphos 48%EC	56.86	60.39	61.57	65.49	y=10.00x+42.88	0.928	5.15
Carbendazim 50%WP	46.27	52.55	60.00	63.14	y=22.10x+15.29	0.997	37.20
Pencycuron 12.5%SC	40.78	48.24	50.20	63.53	y=25.45x+4.39	0.847	61.96
Azoxystrobin 23SC	49.02	51.37	56.08	60.39	y=14.41x+28.01	0.952	33.58
Azoxystrobin 18.2%+Difenoconazole 11.4% SC	76.08	82.35	89.41	100.00	y=29.07x+34.08	0.942	3.53
SEm±	4.00	3.11	3.57	2.35			
CD(P=0.05)	11.98	9.30	10.71	7.03			

18.2% + Difenoconazole 11.4%SC (3.53 ppm). The other test fungicides were in the rank of Triamaphos 48EC < Difenoconazole 25EC < Tricyclazole 34.2% + Propiconazole 10.7% SE< Azoxystrobin 23SC < Carbendazim 50WP < Pencycuron 22.9SC < Tricyclazole 75WP. Jones *et al*. (1987) reported that Propiconazole at 100 ppm concentration resulted 96% reduction of mycelial growth of *R. solani.* The results of the present investigation were similar to the observations made by Biswas (2002), Chahal *et al,* (2003), Sunravadana *et al,* (2007), Bhuvaneswari and Raju (2012). Swamy *et al* (2009) screened some new fungicides against *R. solani* and found that Tricyclazole and Propiconazole had good impact on inhibition of pathogen growth. Haggag and El-Gamal (2012) studied the *in vitro* effect of Propamocarb, Hymexazol, Pencycuron, Flutolanil and Thiophanate methyl against *R. solani* and observed that Hymexazol significantly reduced the mycelia growth of the tested pathogenic fungal isolates, followed by Pencycuron, Propamocarb, Thiophanate methyl and Flutolanil. EC50 is used only to compare the power of fungicides in the control of a specific fungus by identifying the most efficient one having no relation with the actual rate used in field. Hence, present investigation extends the sphere of fungicide options for curative management of blast and sheath blight with triazoles like Cyproconazole 50S, Difenoconazole 25EC and some mixture fungicides like Azoxystrobin 18.2% + Difenoconazole 11.4%SC and Tricyclazole 34.2% + Propiconazole 10.7%SE.

ACKNOWLEDGEMENTS

The author is grateful to Syngenta India Limited for providing the financial assistance and the new fungicide formulations.

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