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Combination fungicides offers substantial potential for management of rice blast disease by *Magnaporthe oryzae*

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Abstract
The eight different combination fungicides *viz*., Flusilazole 12.5% + Carbendazim 25% SC, Azoxystrobin 18.2% + Difenconazole 11.4% w/w SC, Azoxystrobin 11% + Tebuconazole 18.3% w/w SC, Tricyclazole 18% + Mancozeb 62% WP, Zineb 68% + Hexaconazole 4% WP, Trifloxystrobin 25% + Tebuconazole 50% WG, Mancozeb 50% + Carbendazim 25% WS, Fluxapyroxad 6.25% + Epoxiconazole 6.25% EC were evaluated at recommended dosage by poisoned food technique. Highest percent inhibition of mycelial growth of fungus was recorded in Tricyclazole 18% + Mancozeb 62% WP, Mancozeb 50% + Carbendazim 25% WS (100%) on Coimbatore as well as Gudalur blast isolates. Flusilazole 12.5% + Carbendazim 25% SC, Azoxystrobin 18.2% + Difenconazole 11.4% w/w SC, Azoxystrobin 11% + Tebuconazole 18.3% w/w SC and Trifloxystrobin 25% + Tebuconazole 50% WG had exerted maximum control on Gudalur isolate than Coimbatore isolate. These research findings highly demonstrate the efficacy of the combination fungicides and their potential for future rice blast management.

Keywords: Rice blast; *Magnaporthe oryzae;* management; combination fungicides.

Introduction

Rice (*Oryza sativa* L.) is the world's most important crop and staple food for more than a half of the world's population. Global rice consumption has increased by more than 50 million tons, with an average annual growth of nearly 2 percent in the past 7 years. India stands first in area, second in production, followed by China (FAO, 2014) [1]. Rice production is affected by several biotic and abiotic constraints; rice blast disease stands out the most disastrous diseases globally affecting rice production. Blast epidemics happed across various rice growing countries including India, China, Korea, Vietnam and United States to extent of 50% yield loss (Wilson and Talbot, 2009) [2]. *Magnaporthe oryzae* in rice brings forth typical disease symptoms such as leaf blast, nodal blast, neck blast or panicle blast. About 50 species of grass family are infected by *Magnaporthe grisea* (Reddy *et al*., 2004) [3]. Compared to leaf blast, neck blast causes highest yield loss since it affects the panicle directly. An area with high rainfall and cooler climate are sternly affected (Ghatak *et al*., 2013) [4].

The commonly used management approaches to deal with blast are fungicides or to develop resistant varieties. Several plant genes confer rice blast resistance. However, most of these resistant varieties are short-lived and the resistance is broken down due to variable nature of fungal pathogen. In addition, the fungus also gains fungicide resistance by mutating the target genes of fungicides (Kim *et al*., 2003) [5]. Among the methods, chemical control has been withstanding and widely practiced in many countries (Mariappan *et al*., 1995) [6]. With the objective to ascertain the efficacy of various combination fungicides on the management of rice blast disease, the research has been framed.

Materials and methods

Using commercially available combination fungicides *viz*., Flusilazole 12.5% + Carbendazim 25% SC, Azoxystrobin 18.2% + Difenconazole 11.4% w/w SC, Azoxystrobin 11% + Tebuconazole 18.3% w/w SC, Tricyclazole 18% + Mancozeb 62% WP, Zineb 68% + Hexaconazole 4% WP, Trifloxystrobin 25% + Tebuconazole 50% WG, Mancozeb 50% + Carbendazim 25% WS and Fluxapyroxad 6.25% + Epoxiconazole 6.25% EC the mycelial growth of rice blast pathogen was targeted (Table 1). To further evaluate fungicides, poisoned food technique was employed *in vitro* to appraise the growth of mycelium as per recommended dosage. Stringent sterile practices of the laboratory were followed to preclude the accidental contamination. Hermetically sealed sterilized potato dextrose agar medium was appended with an appropriate quantity of fungicides. A pinch of streptomycin sulphate was imparted to forestall bacterial contamination. Upon medium solidification, 5-mm fungal discs of *M. oryzae* were placed in the center of Petri dishes. The hermetically polypropylene sealed plates used were incubated at 25±1 °C for 15 days. Emanated radial mycelial growth was recorded (Hajano *et al*., 2012) [7]. The evaluation of percent inhibition of mycelial growth was performed using the following formula,

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

C = Radial growth of mycelium (Control) in mm
T = Radial growth of mycelium in fungicide amended medium in mm

Statistical analysis

All data from each experiment were subjected to analysis of variance (ANOVA) by using Duncan's Multiple Range-Test (DMRT) at 5% significance (Gomez and Gomez, 1984) [8]. All data were statistically analyzed with SPSS 16.0

Results and Discussion

Effect of eight different fungicides was studied against rice blast pathogen through poison food technique. Mycelial growth of isolates was recorded (Table 2). Two combination fungicides, Tricyclazole 18% + Mancozeb 62% WP and Mancozeb 50% + Carbendazim 25% WS completely inhibited the mycelial growth of Coimbatore and Gudalur isolates (100%). On the contrary, Flusilazole 12.5% + Carbendazim 25% SC, Azoxystrobin 18.2% + Difenconazole 11.4% w/w SC, Azoxystrobin 11% + Tebuconazole 18.3% w/w SC and Trifloxystrobin 25% + Tebuconazole 50% WG had exerted maximum control on Gudalur isolate more rather than Coimbatore isolate. The fungicide Fluxapyroxad 6.25% + Epoxiconazole 6.25% EC recorded the least effective fungicidal action on mycelial growth of Coimbatore and Gudalur isolates. Observation was recorded based on percent inhibition (Table 3, Figure 1). Further grouping of combination fungicides was given as dendrogram based on percent inhibition of mycelial growth of *M. oryzae* (Figure 2)

Table 2: Evaluation of combination fungicide on the growth of *Magnaporthe oryzae* through poisoned food technique

S. No	Treatments	Mycelial growth of *Magnaporthe oryzae* (in cm)*					
		4 days after inoculation		7 days after inoculation		10 days after inoculation	
		Coimbatore	Gudalur	Coimbatore	Gudalur	Coimbatore	Gudalur
1.	Flusilazole 12.5% + Carbendazim 25% SC	1.30^{e} (1.18)	0.00^{a} (0.32)	1.67^{c} (1.34)	0.00^{a} (0.32)	1.97^{e} (1.41)	0.00^{a} (0.32)
2.	Azoxystrobin 18.2% + Difenconazole 11.4% w/w SC	0.83^{b} (0.89)	0.00^{a} (0.32)	1.03^{b} (1.04)	0.00^{a} (0.32)	1.17^{b} (1.09)	0.00^{a} (0.32)
3.	Azoxystrobin 11% + Tebuconazole 18.3% w/w SC	0.97^{c} (0.95)	0.00^{a} (0.32)	1.10^{b} (1.05)	0.00^{a} (0.32)	1.80^{d} (1.30)	0.00^{a} (0.32)
4.	Tricyclazole 18% + Mancozeb 62% WP	0.00^{a} (0.32)	0.00^{a} (0.32)	0.00^{a} (0.32)	0.00^{a} (0.32)	0.00^{a} (0.32)	0.00^{a} (0.32)
5.	Zineb 68% + Hexaconazole 4% WP	0.93^{bc} (0.95)	0.93^{b} (0.95)	1.13^{b} (1.09)	1.07^{b} (1.04)	1.37^{c} (1.18)	1.20^{b} (1.09)
6.	Trifloxystrobin 25% + Tebuconazole 50% WG	1.37^{e} (1.14)	0.00^{a} (0.32)	1.83^{d} (1.37)	0.00^{a} (0.32)	2.27^{f} (1.48)	0.00^{a} (0.32)
7.	Mancozeb 50% + Carbendazim 25% WS	0.00^{a} (0.32)	0.00^{a} (0.32)	0.00^{a} (0.32)	0.00^{a} (0.32)	0.00^{a} (0.32)	0.00^{a} (0.32)
8.	Fluxapyroxad 6.25% + Epoxiconazole 6.25% EC	1.13^{d} (1.09)	1.13^{c} (1.09)	1.57^{c} (1.22)	1.53^{c} (1.22)	1.87^{de} (1.37)	1.90^{c} (1.34)
9.	Control	1.97^{f} (1.41)	2.17^{d} (1.48)	4.57^{e} (2.14)	4.87^{d} (2.21)	5.97^{g} (2.44)	6.27^{d} (2.50)
	SEd	0.03	0.01	0.03	0.01	0.02	0.01
	CD(.05)	0.05	0.02	0.06	0.02	0.05	0.02

*Values are mean of three replications

Figures in parentheses represent square root transformation. Means in a column followed by same superscript letters are not significantly different according to DMRT.

Table 3: Effect of combination fungicides on the percent inhibition of mycelial growth of *M. oryzae*

S. No	Treatments	Percent inhibition of mycelial growth (%)*					
		4 days after inoculation		7 days after inoculation		10 days after inoculation	
		Coimbatore	Gudalur	Coimbatore	Gudalur	Coimbatore	Gudalur
1.	Flusilazole 12.5% + Carbendazim 25% SC	34.01	100.00	63.46	100.00	67.00	100.00
2.	Azoxystrobin 18.2% + Difenconazole 11.4% w/w SC	57.87	100.00	77.46	100.00	80.40	100.00
3.	Azoxystrobin 11% + Tebuconazole 18.3% w/w SC	50.76	100.00	75.93	100.00	69.85	100.00
4.	Tricyclazole 18% + Mancozeb 62% WP	100.00	100.00	100.00	100.00	100.00	100.00
5.	Zineb 68% + Hexaconazole 4% WP	52.79	57.14	75.27	78.03	77.05	80.86
6.	Trifloxystrobin 25% + Tebuconazole 50% WG	30.46	100.00	59.96	100.00	61.98	100.00
7.	Mancozeb 50% + Carbendazim 25% WS	100.00	100.00	100.00	100.00	100.00	100.00
8.	Fluxapyroxad 6.25% + Epoxiconazole 6.25% EC	42.64	47.93	65.65	68.58	68.68	69.70
9.	Control	0.00	0.00	0.00	0.00	0.00	0.00

*Values are mean of three replications

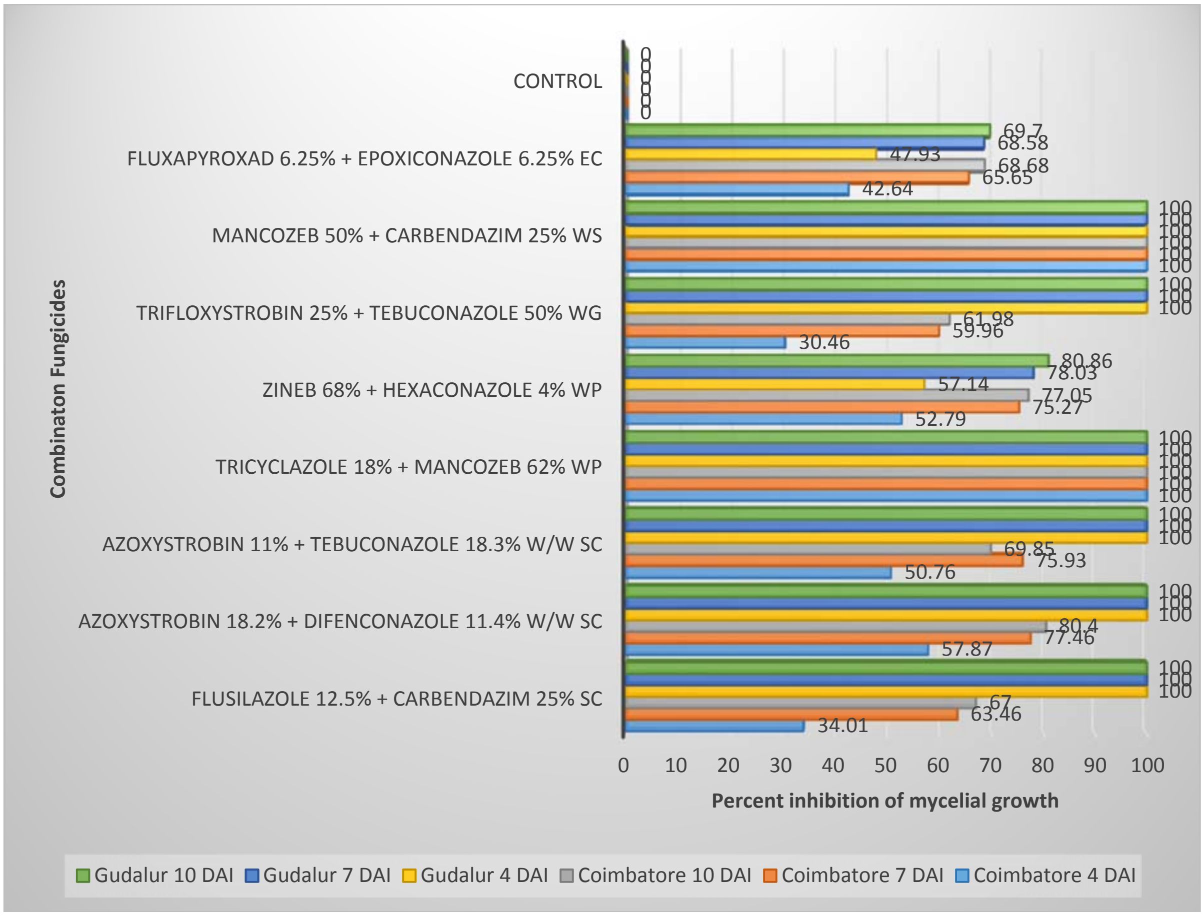


Fig 1: Antifungal efficacy of combination fungicides in the suppression of mycelial growth of *M. oryzae* under *in vitro* condition. Data is presented as percentage inhibition in mycelial growth over control and combination fungicides. Error bars indicate standard deviation obtained from three replicates per treatment. Values in the bar represents the percent reduction of mycelial growth of *M. oryzae* under *in vitro* condition.

In the present investigative study, fungicides Tricyclazole 18% + Mancozeb 62% WP and Mancozeb 50% + Carbendazim 25% WS completely suppressed the growth of the fungus. This is similar to the research findings of Sood and Kapoor (1997) [9], Tirmali *et al.*, (2001) [10], Prabhu *et al.*, (2003) [11], Usman *et al.*, (2009) [12] and Hajano *et al.*, (2012) [7]. Varier *et al.*, (1993) [13] reported that treatment of seeds with tricyclazole @ 4 g/kg seed proved effective upto 40 days of sowing. Fungicides viz., carbendazim, pyroquilon, thiophanate methyl, chlobenthiazone and tricyclazole were found to reduce leaf and neck blast disease severity (Gouramanis, 1995) [14]. Natural infection of rice leaf blast was controlled effectively using two systemic fungicides benomyl and tricylazole at full booting stage (Enyinnia, 1996) [15]. Fungicide tricyclazole was reported effective in controlling leaf blast by Gohel *et al.*, (2008) [16], whereas carbendazim was ascribed to effective control of leaf blast by Kapooria and Hairwadzi (1994) [17], Arun and Singh (1995) [18], Anwar *et al.*, (2002) [19] and Hajano *et al.*, (2012) [7]. Mohan *et al.*, (2011) [20] reported that tebuconazole, tebuconazole + trifloxystrobin were effective against *M. oryzae* causing rice blast.

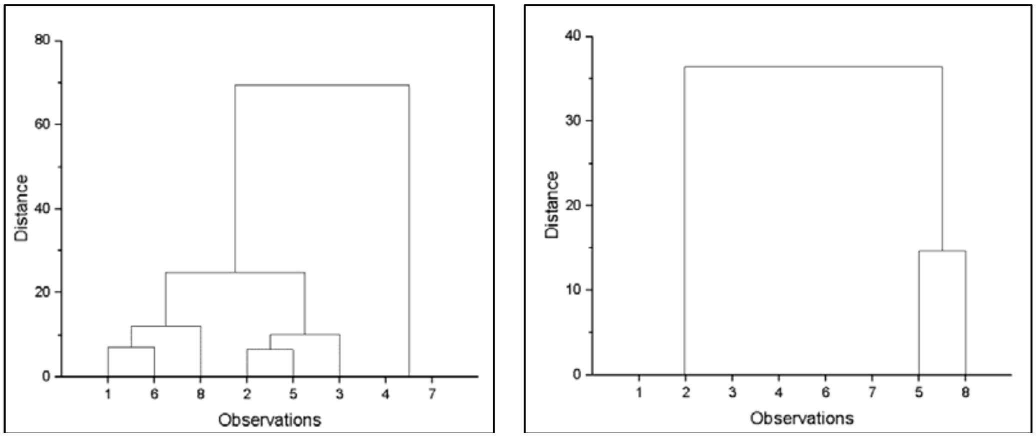
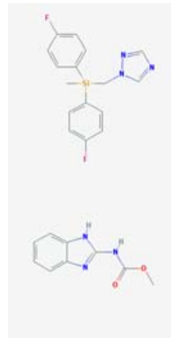
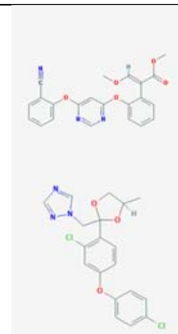
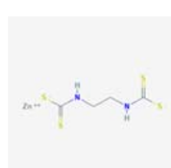


Fig 2: Dendrogram for percent inhibition of mycelial growth of *M. oryzae* by treatment with combination fungicides

Data is presented as observations (various combination fungicides treament) and distance of hierarchical analysis. Observations is represented as 1- Flusilazole 12.5% + Carbendazim 25% SC; 2- Azoxystrobin 18.2% + Difenconazole 11.4% w/w SC; 3- Azoxystrobin 11% + Tebuconazole 18.3% w/w SC; 4- Tricyclazole 18% + Mancozeb 62% WP; 5- Zineb 68% + Hexaconazole 4% WP; 6- Trifloxystrobin 25% + Tebuconazole 50% WG; 7- Mancozeb 50% + Carbendazim 25% WS; 8- Fluxapyroxad 6.25% + Epoxiconazole 6.25% EC

Table 1: List of combination fungicides used in the research

S. No	Common Name	Trade Mark	Chemical Name	2D Structure	Mode of action	Recommended Dose
1.	Flusilazole 12.5% + Carbendazim 25% SC	Lustre 37.5 SE	Bis(4-fluorophenyl)-methyl-(1,2,4-triazol-1-ylmethyl)silane (Flusilazole) and methyl N-(1H-benzimidazol-2-yl)carbamate (Carbendazim)		Flusilazole inhibits ergosterol biosynthesis through direct inhibition of the 14α-demethylation of ergosterol precursors and carbendazim binds to an unspecified site on tubulin and suppresses microtubule assembly dynamic	1.0ml/l
2.	Azoxystrobin 18.2% + Difenconazole 11.4% w/w SC	Amistar Top	Methyl (E)-2-[2-[6-(2-cyanophenoxy) pyrimidin-4-yl]oxyphenyl]-3-methoxyprop-2-enoate (Azoxystrobin) and 1-[[2-[2-chloro-4-(4-chlorophenoxy)phenyl]-4-methyl-1,3-dioxolan-2-yl]methyl]-1,2,4-triazole (Difenconazole)		Azoxystrobin inhibits spore germination and Difenoconazole acts on fungal penetration and haustoria formation and interferes with the biosynthesis of sterols in cell membranes.	1.0ml/l
3.	Azoxystrobin 11% + Tebuconazole 18.3% w/w SC	Custodia	Methyl (E)-2-[2-[6-(2-cyanophenoxy) pyrimidin-4-yl]oxyphenyl]-3-methoxyprop-2-enoate (Azoxystrobin) and 1-(4-chlorophenyl)-4,4-dimethyl-3-(1,2,4-triazol-1-ylmethyl)pentan-3-ol (Tebuconazole)		Azoxystrobin acts as anti-sporulant and Tebuconazole inhibits ergosterol biosynthesis in pathogenic fungus.	1.5ml/l
4.	Tricyclazole 18% + Mancozeb 62% WP	Merger	8-methyl-[1,2,4] triazolo[3,4-b][1,3]benzothiazole (Tricyclazole) and zinc;manganese(2+);N-[2-(sulfidocarbothioylamino)ethyl]carbamodithioate (Mancozeb)		Tricyclazole prevents the fungus from penetrating the plant by inhibition of the melanin pigment formation in appressorium and Mancozeb inactivates the sulphahydral (SH) groups in enzymes of fungi.	2.5g/l
5.	Zineb 68% + Hexaconazole 4% WP	Avtar	Zinc;N-[2-(sulfidocarbothioylamino)ethyl]carbamodithioate (Zineb) and 2-(2,4-dichlorophenyl)-1-(1,2,4-triazol-1-yl)hexan-2-ol (Hexaconazole)		Zineb inactivates the sulphahydral (SH) groups in enzymes of fungi, blocks the metabolism at stages of Kreb Cycle and Hexaconazole is a potent ergosterol	2.5g/l

					biosynthesis inhibitor.	
6.	Trifloxystrobin 25% + Tebuconazole 50% WG	Nativo 75 WG	Methyl (2E)-2-methoxyimino-2-[2-[[(E)-1-[3-(trifluoromethyl) phenyl] ethylideneamino]oxymethyl]phenyl]acetate (Trifloxystrobin) and 1-(4-chlorophenyl)-4,4-dimethyl-3-(1,2,4-triazol-1-ylmethyl)pentan-3-ol (Tebuconazole)		Trifloxystrobin acts as a respiration inhibitor by blocking the electron transfer at fungal mitochondrial membrane and Tebuconazole acts as a demethylation inhibitor (DMI) of fungal sterol biosynthesis.	0.4g/l
7.	Mancozeb 50% + Carbendazim 25% WS	Sprint	Zinc;manganese (2+);N-[2-(sulfidocarbothioylamino)ethyl]carbamodithioate (Mancozeb) and methyl N-(1H-benzimidazol-2-yl)carbamate (Carbendazim)		Mancozeb inactivates the sulphahydral group of enzymes in fungi and Carbendazim acts by disrupting the spindle formation during cell division in fungi.	2.5g/l
8.	Fluxapyroxad 6.25% + Epoxiconazole 6.25% EC	Adexar	3-(difluoromethyl)-1-methyl-N-[2-(3,4,5-trifluorophenyl) phenyl] pyrazole-4-carboxamide (Fluxapyroxad) and 1-[[3-(2-chlorophenyl)-2-(4-fluorophenyl) oxiran-2-yl] methyl]-1,2,4-triazole (Epoxiconazole)		Fluxapyroxad acts as SDHI (Succinate Dehydrogenase inhibitors) and Epoxiconazole acts as a demethylation inhibitor (DMI) of fungal sterol biosynthesis.	1.5ml/l

Conclusion
The present investigation with eight different combination fungicides examined against rice blast pathogen through poison food technique, two fungicides namely Tricyclazole 18% + Mancozeb 62% WP, Mancozeb 50% + Carbendazim 25% WS completely inhibited the mycelial growth of blast isolates (100%). The combination fungicides are better than fungicides with single mode of action, as it provides rigorous control by its diversified mode of action and withstands longer time to pathogens' resurgence in the boom and burst cycle.

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References
1. FAO. Production yearbook of the food and Agriculture Organization, Rome, Italy. 2014.
2. Wilson RA, Talbot NJ. Under pressure: investigating the biology of plant infection by *Magnaporthe oryzae*. Nature Review Microbiology, 2009; 7(3):185-195.
3. Reddy CS, Susheela K, Kapoor AS, Kaundal R, Krishnaiah NV, Mishra B, *et al.* Forewarning Rice Blast in India, Technical Bulletin. Directorate of Rice Research, Rajendranagar, Hyderabad, A.P., India. 2004.
4. 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.
5. Kim ST, Cho KS, Yu S, Kim SG, Hong JC, Han C, *et al.* Proteomics analysis of differentially expressed protein induced by rice blast fungus and elicitor in suspension-cultured rice cells. Proteomics., 2003; 3:2368-2378.
6. Mariappan V, Rajeswari E, Kamalakannan A. Management of rice blast, *Pyricularia oryzae* by using neem (*Azadirachta indica)* amd other plant products. *In:* Mariappan,V. [Ed.] Neem for the Management of Crop Diseases. Associated Publishing Co., New Delhi, India; 1995, 3-10.
7. 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 Journal of Botany., 2012; 44(5):1775-1778.

8. Gomez KA, Gomez AA. Statistical Procedure for Agricultural Research. John Wiley and Sons, New York; 1984.
9. Sood GK, Kapoor AS. Efficacy of new fungicides in the management of rice blast. Plant Dis. Res. 1997; 12:140-142.
10. Tirmali AM, Latake SB, Bendra NJ. Evaluation of new fungicides for control of blast disease of rice. J. Maharashtra Agri. Univ. 2001; 26:197-198.
11. Prabhu, AS, Filippi MC, Zimmermann FJP. Cultivar response to fungicide application in relation to rice blast control, productivity and sustainability. Pesq. Agropec Bras., Brasilia., 2003; 38:11-17.
12. Usman GM, Wakil W, Sahi ST, Yasin S. Influence of various fungicides on the management of rice blast disease. Mycopath, 2009; 7:29-34.
13. Varier M, Maiti D, Shukla VD. Efficacy of combination of fungicide formulations on management of rice-blast (*Pyricularia oryzae*) in rainfed upland. Ind. J. Agric. Sci. 1993; 63:386-389
14. Gouramanis GD. Biological and chemical control of rice blast disease (*Pyricularia oryzae*) in Northern Greece. CIHEAM - Options Mediterraneennes; 1995.
15. Enyinnia T. Effect of two systemic fungicides on the blast control in rainforest zone of Nigeria. Int. J. Pest Manage, 1996; 42:77-80.
16. 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):189-92.
17. Kapooria RG, Hairwadzi E. The effect of carbendazim on the growth of *P. oryzae,* Ghana J. Sci. 1994; 21:33-34.
18. 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; 25:238-42.
19. Anwar A, Bhat GN, Singhara GN. Management of sheath blight and blast in rice through seed treatment. Ann. Pl. Prot. Sci. 2002; 10:285-87.
20. Mohan C, Amrinder K, Sandeep R. *In vitro* evaluation of different fungicides against *Pyricularia grisea*. Pl. Dis. Res. 2011; 26(2):178.