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Evaluation of Some Newly Evolved Fungicides against *Helminthosporium oryzae*, *Alternaria padwickii*, *Fusarium moniliforme*, *Curvularia lunata and Sarocladium oryzae* Causing Grain Discoloration Disease of Rice Under *in vitro* Condition

Sukanta Karmakar[1], Raghunath Mondal[2], Sukanta Dasgupta[1], P. Guha[1], A. K. Mandal[1*]

[1]Department of Seed Science and Technology, Institute of Agricultural Science, University of Calcutta, 51/2, Hazra Road, Kolkata 700019, West Bengal, India
[2]Department of Plant Pathology, B.C.K.V., Nadia, West Bengal, India

*Corresponding Author: akmcu2002[at]gmail.com

Abstract: ***Grain discoloration disease of rice is gradually becoming a serious problem in rice cultivation and seed industry of West Bengal as well as all over India. Discolored rice seeds were collected from famer's field at village Falta, South 24 pargana district, West Bengal. Five pathogens namely Helminthosporium oryzae, Alternaria padwickii, Fusarium moniliforme, Curvularia lunata and Sarocladium oryzae were isolated from diseased seed samples. Seven fungicides namely Trifloxystrobin25% + Tebuconazole50%WG, Pencycuron22.9%SC, Thiafluzamide 24%SC, Carbendazim25% + Mancozeb 50%WS , Tricyclazole18% + Mancozeb 62% WP , Tricyclazole75 % WP and Propiconazole13.9%+ Difeconazole13.9% EC were tested against these five pathogens under in vitro condition. Among these fungicides, five fungicides namely Trifloxystrobin 25%+Tebuconazole50%WG , Carbendazim25%+ Mancozeb 50%WS, Tricyclazole18%+Mancozeb 62% WP, Tricyclazole75 % WP , Propiconazole13.9%+, Difeconazole13.9% EC had shown complete inhibition of growth of all the test pathogens.***

Keywords: Fungicide, Grain Discoloration, Rice, Poison Food Technique, Percent Growth inhibition.

1. Introduction

Rice is the staple food for over half of the world population and it is ranked as the number one human food crop in world (Itani *et. al*., 2002; Sabudhi *et. al*., 2012). More than 90% world rice grown and consumed in Asia (Tyagi *et. al*., 2004) India occupies second position in production of rice in world and it is grown in an area of 44.6 mha. In India, West Bengal is a leading rice growing state, here rice is grown in all three seasons namely Aus, Aman and Boro with an area of 56,00,000 ha and average yield is almost 4100 kg/ha. In West Bengal disease is a major constrains which causes reduction in grain yield and seed yield. The crop suffers with a number of diseases caused by Fungi, Bacteria, Viruses, Nematodes and Mycoplasma like organism. Besides major diseases, grain discoloration is another disease which is becoming a major disease in rice cultivation and emerging as a threat to seed industry.

Grain discoloration of rice is a complex disease which occurs mainly due to infection of certain micro-organisms. Other than climatic and nutritional factors, among the micro-organisms, fungi play a major role by infecting glumes, kernels or both. Two groups of fungi are associated in grain discoloration of rice (Ou, 1985). One group is field fungi, more or less parasite and infects grains before harvest like *Helminthosporium oryzae, Pyricularia oryzae, Alternaria* sp., *Fusarium moniliforme, Nigrospara* sp., *Curvularia* sp., *Phoma* sp., *Sarocladium* sp., *Epicoccum* sp., etc. Other groups are storage moulds and saprophytes develop after harvest. They are mainly species of *Aspergillus, Penicillium, Mucor, Rhizopus* etc. Baldacci and Corbetta (1964) reported that in addition to fungi, bacteria like *Xanthomonas sp., Pseudomonas fuscovaginae, Pseudomonas avenae, Pseudomonas glumae* etc. are also associated with discoloured rice seed. All these micro-organism affect the seeds in various ways. When it is occurred by field fungi, it results in seedling mortality, reduction in germination and seedling vigour. (Das and Narain, 1988; Zulkifi *et. al*., 1991; Bag, 2007). When infections are occurred in storage by storage moulds, viability and grain quality of rice is reduced due to toxins are produced by them.

Regarding management of the disease different workers have worked with different fungicides like Benomyl 50% WP or Mancozeb 80%, WP (Mishra et. al., 1994); Dithane M-45, Ridomil, Carbendazim (Arshad *et. al.*, 2009); Carbendazim 50%, WP and Carbendazim 64% + Mancozeb 8% WP (Bag *et al*., 2010). However, more investigations are required for this disease, otherwise it will be a serious problem in future.

So, considering the importance of the disease, the present investigation was carried out to evaluate different newly

evolved fungicides against five major fungi, *Helminthosporium oryzae*, *Alternaria padwickii, Fusarium moniliforme, Curvularia lunata* and *Sarocladium oryzae.*

2. Materials and Methods

Identification and isolation of pathogen: Rice panicles with discoloured seeds were collected from Rice Research Station, Chinsurah and farmers'plot at Nadia and South 24 Parganas district at different times. The seeds were separated from the panicle and kept in dry petridishes. Then the seeds were surface sterilized with 2% Sodium hypochlorite solution for 30 seconds and then washed with sterilized distilled water. Ten seeds were placed on petriplates following standard Blotter method and incubated at 28˚c for 7 days according to International Seed Testing Association.

The pathogens were initially identified morphologically on the basis of colony colour shape and size. Then microscopic examination were done and pathogens were confirmed on the basis of mycelia structure, spore shape and size as described in the Technical bulletin on Seed bone diseases and seed health testing of rice (Ellis, 1980; Agarwal, 1989). Then identified pathogens were isolated and pacified in Potato Dextrose Agar (PDA) slants for further study.

Maintenance of Isolated Pathogens: Pure culture of five seed borne fungal pathogens were namely *Curvularia lunata, Helminthosporium oryzae, Alternaria padwickii, Sarocladium oryzae* and *Fusarium moniliforme* were maintained and fungal growths were prepared on the PDA medium in petri plate for further study.

Poison food Technique: The effect of seven fungicides namely Trifloxystrobin 25% + Tebuconazole 50%WG , Pencycuron 22.9%SC, Thiafluzamide 24% SC, Carbendazim25% + Mancozeb 50%WS, Tricyclazole 18% +Mancozeb 62% WP, Tricyclazole75 % WP, Propiconazole 13.9% + Difeconazole 13.9% EC were tested against five pathogens (*C. lunata, H. Oryzae, A. padwickii, S. Oryzae* and *F. Moniliforme*.) following poison food technique. We have selected the fungicides used in the experiment on the basis of their availability in the market in commercial form. The fungicides at recommended doses were added and mixed thoroughly with the PDA medium after sterilization when the temperature of the medium was 54˚C (approx) PDA medium without any fungicide was used as control. Five no. of replications were made for each fungicide and for control treatment. Then five (5mm) diameter agar disc of seven (7) days old culture was transferred in the centre of each PDA plate and incubated at 27± 2˚c. The radial growth (mm) of each fungus was recorded when the test fungus mycelia covered whole plate (i,e 90 mm) in control plate.

The growth inhibition percentage was calculated by using the formula given by Vincent (1947).

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

Where, C = Colony diameter in control plate in mm.

T = Colony diameter in treatment plate in mm.

The list of fungicides with nature, trade name and sources are given below:

Sl. No.	*Active Ingredients*	*Nature*	*Trade Name*	*Source*
1.	Tubeconazol 50 % + Triflioxystrobin 25% WG	Systemic	Nativo	Bayer crop science Ltd., Cambridge
2.	Pencycuron 22.9 % SC	Contact	Monceren	Bayer crop science Ltd., Cambridge
3.	Thifluzamide 24 % SC	Systemic	Pulsor	Insecticide India
4.	Carbendazim 25 % + Mancozeb 50 % WS	Contact & Systemic	Sprint	Indofil chemicals Ltd.,Mumbai
5.	Tricyclazole 18 % + Mancozeb 62 % wp	Contact & Systemic	Merger	Indofil chemicals Ltd.,Mumbai
6.	Tricyclazole 75% WP	Systemic	Baan	Indofil chemicals Ltd.,Mumbai
7.	Propiconazole 13.9 % + Difenoconazole 13.9 % EC	Systemic	Taspa	Syngenta

3. Results and Discussion

In this study, it was revealed that fungicides namely Trifloxystrobin 25%+Tebuconazole50%WG, Carbendazim25%+ Mancozeb 50%WS, Tricyclazole18%+Mancozeb 62% WP, Tricyclazole75 % WP, Propiconazole13.9%+ Difeconazole13.9% EC inhibited the growth of all five fungi *Curvularia lunata, Helminthosporium oryzae, Alternaria padwickii, Sarocladium oryzae* and *Fusarium moniliforme* completely. Whereas other two fungicides namely Pencycuron 22.9%SC, Thiafluzamide 24% SC inhibited the growth of *Curvularia lunata, Helminthosporium oryzae, Alternaria padwickii, Sarocladium oryzae* and *Fusarium moniliforme* i.e. 30, 13.3, 30, 7.14, 10.00 and 46.6, 12.2, 46.6, 7.16, 4.44 percent respectively (Table – 1 and Table – 2).

Table 1: In vitro evaluation of newly evolved fungicides against *Curvularia lunata, Helminthosporium oryzae and Alternaria padwiki*

Sl. No.	*Name of fungicide (Treatment)*	*Doses (per 100 ml)*	*Curvularia lunata*		*Helminthosporium oryzae*		*Alternaria padwickii*	
			Radial growth (mm)	*Growth inhibition (%)*	*Radial growth (mm)*	*Growth inhibition (%)*	*Radial growth (mm)*	*Growth inhibition (%)*
1.	Trifloxystrobin 25%+ Tebuconazole50%WG	0.1 g	0	100	0	100	0	100
2.	Pencycuron 22.9%SC	0.15 ml	63	30	78	13.3	81	10
3.	Thiafluzamide 24% SC	0.075 ml	48	46.6	79	12.2	41	54.4
4.	Carbendazim25%+Mancozeb 50% WS	0.25 g	0	100	0	100	0	100
5.	Tricyclazole18%+Mancozeb 62% WP	0.25 g	0	100	0	100	0	100
6.	Tricyclazole75 % WP	0.05 g	0	100	0	100	0	100
7.	Propiconazole13.9% + Difeconazole13.9% EC	0.075 ml	0	100	0	100	0	100
8.	Control	-	90	0	90	0	90	0
	CD (0.05)	-	1.067	-	1.054	-	1.138	-

Table 2: *In vitro* evaluation of some newly evolved fungicides against *Sarocladium oryzae and Fusarium moniliforme*

Sl. No.	*Name of fungicide (Treatment)*	*Doses (per 100 ml)*	*Sarocladium oryzae*		*Fusarium moniliforme*	
			Radial growth (mm)	*Growth inhibition (%)*	*Radial growth (mm)*	*Growth inhibition (%)*
1	Trifloxystrobin 25%+ Tebuconazole50%WG	0.1 g	0	100	0	100
2	Pencycuron 22.9%SC	0.15 ml	83.57	7.14	81	10
3	Thiafluzamide 24% SC	0.075 ml	83.55	7.16	86	4.44
4	Carbendazim25% + Mancozeb 50% WS	0.25 g	0	100	0	100
5	Tricyclazole18% + Mancozeb 62% WP	0.25 g	0	100	0	100
6	Tricyclazole75 % WP	0.05 g	0	100	0	100
7	Propiconazole13.9% + Difeconazole13.9% EC	0.075 ml	0	100	0	100
8	Control	-	90	0	90	0
	CD (0.05)	-	0.964	-	1.009	-

Gupta *et al.* (2013) worked with seven fungicides (Propiconazole, Hexaconazole, Tricyclazole, Carbendazim, Triadimefon, Mancozeb and Azoxystrobin) against *Bipolaris orzae* under *in vitro* condition by poison food technique and observed that Propiconazole was most effective fungicide at different concentrations. Other fungicides like Mancozeb, Tricyclazole had also some effect in growth inhibition at high concentration (250 ppm).

In another *in vitro* study Hazano *et al.* (2012) used with five fungicides (Thiophanate-methyl, Carbendazim, Fosesetyl-aluminium, Mancozeb and Copper oxychloride) against *Magnaporthe oryzae*, the rice blast pathogen and reported that Mancozeb appeared as highly effective fungicide in inhibiting mycelial growth of the fungus completely.

Under *in vitro* evaluation with fourteen fungicides of different groups against *Rhizoctonia solani*, Srinivas *et al.* (2013) reported that Metalaxyl (0.1%), Mancozeb (0.1%), Tricyclazole (0.1%), Thiophenate methyl (0.1%) and Carbendazim+ Mancozeb (0.1%) were proved to be most effective in inhibiting the growth of the fungus. In the present experiment, it was found that five chemical fungicides namely Trifloxystrobin25% + Tebuconazole50%WG, Carbendazim25%+ Mancozeb 50%WS , Tricyclazole18% +Mancozeb62% WP, Tricyclazole75 % WP and Propiconazole13.9% + Difeconazole13.9% EC were equally effective in inhibiting the growth of *Helminthosporium oryzae, Alternaria padwickii, Curvularia lunata, Fusarium moniliforme* and *Sarocladium oryzae* under *in-vitro* condition.

Therefore, on the basis of the results, all the aforesaid fungicides may be safely used for the control of *Helminthosporium oryzae, Alternaria padwickii, Curvularia lunata, Fusarium moniliforme* and *Sarocladium oryzae* which are responsible for the discoloration of rice grain.

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