In Vitro Exploration of Fungicides, Phytoextracts And Bio-Agents Against *Pyricularia grisea* (Cooke) Causing Blast of Rice (*Oryza sativa* L.)

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Abstract - The severe incidence of blast disease of rice incited by Pyricularia grisea (Cooke) was observed on rice crop cultivated at the Department of Agronomy, Dr. Balasaheb Sawant Konkan Krishi Vidyapeeth, Dapoli, Dist. Ratnagiri (M.S.). Among the different fungicides evaluated in vitro, Tricyclazole 75% WP (0.1%), Thiophanate methyl 70% WP (0.1%) and Carbendazim 50% WP (0.1 %) completely inhibited growth of P. grisea. Among different plant extracts tested against P. grisea, the maximum mycelial inhibition (77.20 %) of the test fungus was achieved due to leaf extract of Neem followed Tulsi (75.01 %). Among bioagents, maximum inhibition of the P. grisea was achieved due to Trichoderma harzianum followed by Pseudomonas fluorescens and T. viride.

Key words:- Blast, Pyricularia grisea, Tricyclazole, Copper oxychloride, Carbendazim, Thiophanate methyl, phytoextracts, bio-agents.

INTRODUCTION

Rice (*Oryza sativa* L.) is the most important staple food grain crop of the world which constitutes the principle food for about 60 percent of the world's population. It contributes 43 percent of total food grain production and 46 percent of total cereal production in India. India is the world's second largest rice producer and consumer next to China. About 90 per cent of rice grown in the world is produced and consumed in Asian countries. In the world, rice is cultivated on about 163.1 million hectares of area with total production of 722.5 million tones and productivity is 4.4 tones ha^{-1}. Rice has unique position in Indian economy. India ranks second in rice production followed by China (Anonymous, 2012). In Maharashtra state, rice is cultivated on 15.13 lakh hectares area in almost all four regions *viz*.,Vidharbha (7.95 lakh ha.), Konkan (3.83 lakh ha.), Western Maharashtra (3.23 lakh ha.) and Marathwada (0.12 lakh ha.) with annual production of 41.71 lakh tonnes unmilled (brown rice) and 28.78 lakh tonnes milled rice. Highest productivity was recorded in Konkan region *i.e*. 2.75 tonnes ha^{-1} milled rice and 3.83 tonnes ha^{-1} unmilled (brown rice) with total production of 15.26 lakh tonnes unmilled (brown rice) and 10.53 lakh tonnes milled rice from 3.83 lakh hectares area in the Konkan regions (Anonymous, 2014).

Maximum diseases of rice have been reported from different parts of the world which are obstacle to get maximum production of this crop. Blast disease, the most dreaded disease of rice, is of great economic importance and responsible for the failure of many popular varieties in different countries. The present investigations was undertaken to test *in vitro* efficacy of fungicides, plant extracts and bio-agents against *P. grisea* for its effective management.

MATERIAL AND METHODS

In vitro evaluation of fungicides against *P. grisea* :

Eight different fungicides were tested for their efficacy against the test pathogen by using Poisoned Food Technique (PFT) (Nene and Thapliyal, 1993). Oat meal agar medium was used as basal medium and distributed 100 ml aliquots in each 250 ml Erlenmeyer conical flasks, which were sterilized at 15 lbs psi for 20 minutes. The quantity of each fungicide for each concentration was calculated for 100 ml medium, separately. The weighed quantity of the fungicides were added in molten oat meal agar at 40 ± 2 ^{0}C and mixed thoroughly. Such poisoned medium was then poured into sterilized Petri plates and allowed to solidify. The culture discs of 5 mm diameter were cut from 7 day old culture of the test pathogen with the help of sterile cork borer. A culture disc was transferred aseptically to the centre of the plate already poured with poisoned medium. The plates without fungicide were also inoculated with fungal culture disc which served as control. The plates were incubated at 27 ± 2 ^{0}C in the BOD incubator. Three replications per treatment were maintained. The observations for colony diameter and sporulation were recorded until whole of the plate in control treatment was fully covered with fungal growth.

Per cent inhibition of growth of the test fungus was calculated by following formula (Horsfall, 1956).

$$X = \frac{Y - Z}{Y} \times 100$$

Where, X = Per cent inhibition

Y = Growth of fungus in control (mm)

Z = Growth of fungus in treatment (mm)

Efficacy of phytoextracts against *P. grisea* :

The plant extracts of neem, tulsi, sadafuli, ginger, turmeric, garlic, onion and glyricidia were used during the studies. Aqueous phytoextracts were obtained as per the method described by Bhatti (1988). Hundred gram fresh plant materials were washed thoroughly with sterile distilled water and ground well in 100 ml distilled sterile water. The macerate was filtered through sterilized double layered muslin cloth and centrifuged at 4000 rpm for 5 minutes. After centrifuging, the supernatant was then filtered through filter paper. Extracts thus obtained were passed through Sintered glass filter separately to avoid bacterial contamination. This formed the standard plant extract solution (100 %).

The effect of plant extracts on mycelial growth was studied by 'Poisoned Food Technique' (Nene and Thapliyal, 1993). All the plant extracts were tested at 10 per cent concentration against the test pathogen using oat meal agar as a basal medium. Three replications of each treatment were maintained. The observations on colony diameter of the fungus were recorded when Petri plate in control treatment was fully covered with mycelial growth.

Efficacy of bioagents against *P. grisea* :

The laboratory experiment was conducted with bioagents *viz*., *Trichoderma harzianum, T. viride* and *Pseudomonas fluorescens* (bacterial bio-agent) against the causal fungus *P. grisea*.

The trial was conducted by using dual culture technique in which the fungal discs of 5 mm diameter were placed in a Petri plate in such a way that both the organisms in the plate will get equal opportunity for growth. For testing of *P. fluorescens* streaking method were followed.

Each treatment was replicated three times. The plates were then incubated at room temperature (27 ± 2°C) for seven days. The observations on colony diameter of the test fungus were recorded seven days after inoculation when Petri plate in control treatment was fully covered with mycelial growth of the test pathogen.

Per cent inhibition of growth of the test fungus was calculated by using formula as mentioned previously (Horsfall, 1956).

RESULTS AND DISCISSION

***In vitro* evaluation of fungicides against *P. grisea*:**

Among the different fungicides tested under in vitro conditions, Tricyclazole 75% WP (0.1%), Thiophanate methyl 70 % WP (0.1%) and Carbendazim 50 % WP (0.1 %) were completely inhibited the mycelial growth and conidia formation of P. grisea, which was significantly superior to rest of the treatments.

It was followed by Mancozeb (87.67%), Copper hydroxide (83.07%), Copper oxychloride (80.69%) and Zineb (80.58%). Captan (72.77%) was found to be the least effective fungicide. The findings are in agreement with those reported by Hossain and Kulkarni (2001), Joshi and Mandokhot (2002), Vijay (2002) and Bhojyanaik et al. (2014), who also reported Tricyclazole, Thiophanate methyl, Carbendazim, Mancozeb, and Copper hydrocide the most effective fungicides against P. grisea.

***In vitro* evaluation of phytoextracts against *P. grisea*:**

All the plant extracts tested at 10 per cent concentration were found significantly effective in inhibiting the mycelial growth of P. grisea. Maximum per cent inhibition of P. grisea over control was achieved due to leaf extract of Neem (77.20%) and was significantly superior over rest of the treatments and was at par with leaf extract of Tulsi with 75.01 per cent inhibition of test pathogen over control. These were followed by extracts of Glyricidia, Garlic, Turmeric, Ginger and Sadafuli which showed 60.21, 59.47, 56.59, 37.26 and 36.31 per cent inhibition, respectively over control. Onion was found to be least effective phytoextract in inhibiting the mycelial growth of the test pathogen which recorded only 33.22 per cent inhibition over control. The results on effectiveness of plant extracts under study are in conformity with those reported by Jayashree et al. (2000) and Kamalakannan et al. (2001). Jamal et al. (2012) reported that each 50 gm of neem leaves, Calatropis leaves and garlic bulbs were used 1, 2 and 4 ml /15ml, separately. The higher concentration (4 ml/15ml) of neem extract was highly effective in checking the mycelial growth of P. grisea (20.58 mm). Hossain and Kulkarni (2001) reported that Azadirachta indica was most effective phytoextracts to inhibit the growth of P. grisea. Kapoor et al. (2014) found that Ocimum sanctum (Tulsi) and Aegle marmelos (Bael) were very effective in blast control.

***In vitro* evaluation of bio-agents against *P. grisea* :**

The present study revealed that Trichoderma harzianum showed maximum (83.69%) inhibition of P. grisea over control which was followed by Pseudomonas fluorescens (77.03%). The treatment with T. viride resulted in 76.67 and 64.81 per cent inhibition of P. grisea over control at periphery and at center, respectively. The above findings are in close conformity with the results of Agrios (1988) and Soytong (1991) who recorded maximum inhibition of P. grisea by T. harzianum. Similarly, Hossain and Kulkarni (2001), who tested the efficacy of, T. viride, T. harzianum, T. koningii, Gliocladium virens and P. fluorescens against P. grisea which was prominently, inhibited the mycelial

growth of P. grisea under laboratory conditions following the dual culture technique. Ali et al. (2002) studied the highest severity of leaf blast was found due to treatment by T. harzianum followed by T. viride and P. fluorescens. The present findings are also in line with the results of Jamal et al. (2012).

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Table 1. *In vitro* efficacy of fungicides on *P. grisea*

Sr. No.	Fungicide	Conc. (%)	Mean colony dia.(mm)	Per cent inhibition
T_1	Copper oxychloride 50% WP	0.25	17.36	80.69
T_2	Mancozeb 75% WP	0.25	11.56	87.67
T_3	Copper hydroxide 77% WP	0.2	15.23	83.07
T_4	Tricyclazole 75% WP	0.1	0.00	100.00
T_5	Captan 50% WP	0.2	24.5	72.77
T_6	Thiophanate methyl 70% WP	0.1	0.00	100.00
T_7	Zineb 75% WP	0.25	17.46	80.58
T_8	Carbendazim 50% WP	0.1	0.00	100.00
T_9	Control	-	90.0	-
	S.Em ±		**0.24**	
	C. D. at 1%		**0.98**	

Table 2. Effect of plant extracts on growth of *P. grisea*

Sr. No.	Common name	Botanical name	Conc. (%)	Mean colony diameter (mm)*	Per cent inhibition
T_1	Garlic	*Allium sativum* L.	10	36.46	59.47
T_2	Onion	*Allium cepa* L.	10	60.09	33.22
T_3	Sadafuli	*Catharanthus roseus* L.	10	57.31	36.31
T_4	Turmeric	*Curcuma longa* L.	10	39.05	56.59
T_5	Tulsi	*Ocimum sanctum* L.	10	22.48	75.01
T_6	Neem	*Azadirachta indica*	10	20.51	77.20
T_7	Glyricidia	*Gliricidia maculata*	10	35.80	60.21
T_8	Ginger	*Zingiber officinale*	10	56.46	37.26
T_9	Control	-	-	90	-
		S.Em ±		**0.49**	
		C.D. at 1%		**1.98**	

Table 3. Effect of bio-agents on growth of *P. grisea*

Treatment No.	Placement details	Average colony diameter (mm)	Per cent inhibition over control
T_1	*Th* *Pg* *Th* *Th*	14.67	83.69
T_2	*Pg* *Th* *Pg* *Pg*	30	66.67
T_3	*Pf* *Pg* *Pf* *Pf*	20.67	77.03
T_4	*Pg* *Pf* *Pg* *Pg*	81.34	9.62
T_5	*Pg* *Tv* *Pg* *Pg*	31.67	64.81
T_6	*Tv* *Pg* *Tv* *Tv*	21	76.67
T_7	Control	90	-
	S. Em ±	**1.83**	
	C. D. at 1 %	**7.70**	

Where,

Pg = Pyricularia grisea *Tv= Trichoderma viride*

Th= T. harzianum *Pf= Pseudomonas fluorescen*