IN VITRO STUDY OF " The Rice Blast Caused By Pyriculuria Grisea "

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ABSTRACT

The present study was proposed with following objectives , Collection of diseased samples, isolation and identification of the causal agent. To determine the optimum conditions for the growth of the pathogen and development of the disease. To find out the efficacy of certain plant extracts, natural products and bio agents against *Pyricularia grisea.* To find out efficacy of eco friendly products against the growth and yield parameters of the host. It was found that maximum radial growth of mycelium of Pyricularia grisea was supported by potato dextrose agar (PDA) medium whereas, higher sporulation i.e 3.2x106 and 6.1x106 conidia/ml was produced on oat meal agar (OMA) medium and media M1 (Arachne grass + Sucrose + Oatmeal powder) respectively. Blast was artificially created at nursery stage under field conditions by spraying conidial suspension of P. grisea and covering of inoculated seedlings with perforated plastic bottles to maintain high relative humidity. At tillering stage disease was created artificially by inoculating plants with inoculum concentrations i.e 1x105 and 2x107 coinidia/ml. Inoculum concentration of 2x107 coinidia/ml resulted 83.0 per cent average leaf blast severity, whereas 1x105 conidia/ml resulted in 76.0 per cent average leaf blast severity as compared to control where average leaf blast severity recorded was 14.0 per cent. P. grisea produced characteristic rice blast symptoms on different weeds viz. Arachne racemosa, Digitaria sanguinalis, Dactyloctenuim aegyptiacum, Echinochloa crusgalli, Echinochloa colonum, Cyperus difformis and Cypreus iria. Studies on survival revealed that both infected seed and infected straw serve as source of survival but infected crop residue was found to serve as more efficient source for overwintering the inoculum as compared to seed.

INTRODUCTION

Rice blast disease is caused by *Magnaporthe grisea* (Hebbert) Barr (Anamorph: *Pyricularia grisea* (Cooke) Sacc, which is also known as rice blast fungus, rice seedling blight, rice rotten neck, oval leaf spot of graminea, blast of rice, pitting disease, Johnson's spot and rye grass blast. The perfect stage of *Pyricularia grisea* was earlier named as *Ceratosphaeria oryzae* (Hebert, 1971). Later Yaegashi and LHel>ert (1976) suggested the genus *Magnaporthe.* Yaegashi and Udagawa (1978) finally proposed *M. grisea* as a perfect stage *of Pyricularia grisea* (cooke.) Sacc. instead of *Ceratosphaeria grisea.*

The mycelium consists of septate, uninucleate, branched hyphae. However, as the fungus gets older, the hypha become brown. Generally, growth of the pathogen is relatively more on upper surface making the spot more dark on upper side. Conidiophores are simple, septate, basal portion being relatively darker. Conidia are pyriform in shape and hyaline in colour, produced acrogenously, one after another. Conidia are three celled, the middle cell being much wider and darker, and end cell germinates giving out germ crop loss caused by plant diseases. Therefore, extensive searches for biofungicides that are environmentally safe and easily biodegradable have been carried out during the last two decades (Gnanamanickam, 2002).

REVIEW OF LITERATURE

Boza et al . (2006) studied "the race pattern of nine isolates of P. grisea . The seedlings of thirty three rice varieties were spray inoculated with rice blast pathogen at 2.0×105 at 3 - 4 leaf stage. Two per cent Tween 20 was added to 50- 100 ml of inoculum as a sticking agent. After inoculation the plants were placed in a dew chamber at 100% relative humidity at approximately 21-22 0 C for 24 h. The plants were then transferred to green house at

approximately 28 -30 0 C for 6 - 7 days and scored for disease reaction using a qualitative and quantitative rating scale from 0 -9".

Evaluation of fungicides:

Shiba and Nagata (1981) reported that "tricyclazole inhibited mycelial growth, conidial germination and appressorial formation of P. oryzae at concentrations less than 125 ppm. In main field it completely protected the plants from the disease by foliar application at as low as 10 - 20 ppm".

Carreres et al . (1986) evaluated "four fungicides (thiabendazole, prochloraz, isoprothiolane and tricyclazole) against blast and found isoprothiolane reduced leaf blast and tricyclazole and isoprothiolane were effective in reducing neck and node infection when the fungicides were applied as fortnightly foliar spray safter disease symptoms appeared (post panicle initiation to flowering)".

MATERIALS AND METHODS

Chemicals

All the chemicals and reagents used in this thesis work were of Analytical Reagent (A.R) quality unless otherwise special mention was made and sterile distilled water was used throughout the studv.

Potato dextrose agar (PDA) medium

Potato (peeled)	-	250.0 g
Agar agar	-	15.0g
Dextrose	-	20.0 g
Distilled water	-	1000.0 ml

The Potato Dextrose Agar (PDA) was used for this experiment. The pH of both solid medium and broth was adjusted to levels of 5, 6, 7, 8 and 9.

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

Oat meal agar (OMA) medium

Oatmeal	-	30.0 g
Agar agar	-	20.0 g
Distilled water	-	1000.0 ml

Five Petri plates having 20 ml of OMA medium were inoculated with a 5 mm diameter culture block of ***P. grisea*** Cav Oat Meal agar medium was used as basal medium to study the influence of carbon compounds on growth and sporulation. Various carbon compounds, viz. glucose, dextrose, sucrose, fructose, galactose, starch and maltose were tested.

METHODS

- Efficacy of various botanicals against the mycelial growth and spore germination of Pyricularia grisea (Poisoned Food Technique)

- Efficacy of newly evolved fungicides in paddy challenges inoculated with P. grisea causing blast disease

RESULT

Table 1. Effect of Different Media on the Radial Growth of *Pyricularia grisea*

Media	Growth (mm)					Sporulation
	3 DAI	6 DAI	9 DAI	12 DAI	15 DAI	
Potato Dextrose Agar (PDA)	26.7[b]	43.7 [b]	71.00 [b]	789.8 [b]	87.3 [b]	+
Oat Meal Agar (OMA)	28.5[a]	45.5 [a]	71.3 [a]	83.2[a]	90 [a]	++++
Corn Meal Agar (CMA)	14.1[f]	26.0[f]	59.33[d]	71.7[d]	79.5[d]	+++
Czapek'sdox Agar (CMA)	15.1[e]	29.3[d]	47.33[e]	54.3[e]	62.5[f]	+
Carrot Dextrose Agar (CDA)	19.0[d]	28.1[e]	36.6[f]	48.1[f]	69.6[e]	++
Rice Leaf Dextrose Agar (RLDA)	23.6[c]	43.3[e]	68.4[c]	75.2[c]	84.1[c]	++++

* Values are expressed as means + S.d. for three replications in each group.
Values not sharing a common superscript differ significantly at P<0.05 (DMRT

: Efficacy of various botanicals against the mycelial growth and spore germination of Pyricularia grisea (Poisoned Food Technique)

Sl. No.	Local name of plants	Botanical name	Colony diameter (mn)	% growth inhibition overn control	Spore germination (%)	Germination inhibition (%)
1.	Neem	Azadirachata indica	32.5^{c}	63.8^{c} (53.0)	28.3^{e} (32.1)	69.2^{c} (56.3)
2.	Henna	Lawsonia inermis	18.9^{b}	79.0^{b} (62.7)	18.8^{f} (25.7)	79.5^{b} (63.1)
3.	Yerukka	Calotropis gigantean	64.1^{e}	28.7^{c} (32.4)	87.8^{b} (43.74)	48.0^{e} (43.8)
4.	Seemaikaruvelam	Prosopis julifera	72.9^{f}	19.0^{f} (25.8)	58.8^{b} (50.7)	35.0^{f} (36.3)
5.	Arugampul	Cynodon dactylon	61.3^{d}	31.8^{d} (34.32)	38.4^{d} (38.3)	58.2^{d} (49.7)
6.	Eucalyptus	Eucalyptus globulus	5.6^{a}	93.7^{a} (75.5)	8.9^{a} (70.6)	90.3^{a} (72.8)
7.	Control	Water	90.0	-	92.0	-

*** Values are expressed as means ± S.D. for three replications in each group.**
Values not sharing a common superscript differ significantly at P<0.05 (DMRT).

Efficacy of newly evolved fungicides in paddy challenges inoculated with P. grisea causing blast disease

Sl. No.	Treatment	Dose (g/lit, ml/lit of water)	PDI (%)	% disease control
T_1	Trifloxystrobin 25% + Tebuconazole 505 (Nativo 75 WG)	1.0	13.7a (21.6)	43.60a
T_2	Pyriquilon (Fongorene 50 WP)	1.0	24.7e (29.7)	27.67e
T_3	Azoxystobin (Amistar 25 SC)	1.0	28.5f (32.2)	15.92b
T_4	Tricyclazole (Beam 75 WP)	0.6	16.7b (24.0)	37.33b
T_5	Carbendazim (Bavistin 50 WP)	1.0	21.8d (24.7)	22.45d
T_6	Propiconazole (Tilt 25 EC)	1.0	19.6c (26.2)	31.59c)
T_7	Inoculated Control	-	38.6g (38.3)	-

*** Values are expressed as means ± S.D. for three replications in each group.**
Values not sharing a common superscript differ significantly at P<0.05 (DMRT).

CONCLUSION

Pyricularia grisea was grown on six different solid media. Among that highest mycelial growth and spoliation was observed on Oat meal agar.

Out of five different pH levels tested, best radial growth and vegetative growth of the fungus was achieved in pH 6.

The pathogen was grown in medium supplemented with different carbon sources. Among that such supported maximum mycelial growth.

Various plant extracts tested against Pyricularia grisea Cav. by poisoned food technique Among that least mycelial growth of was recorded with the extract of eucalyptus.

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