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EFFECT OF BIO PRODUCTS AND BIO INOCULANTS AGAINST *PYRICULARIA GRISEA* THE INCITANT OF BLAST DISEASE IN RICE

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Abstract

Among the fungal diseases in rice, blast incited by *Pyricularia grisea* is a major disease. *In vitro* studies were conducted to control the pathogen by using natural products *viz.,* plant extracts, animal excrements and bio control agents. Among 10 commonly available botanicals, tested for their efficacy against *P. grisea* by poisoned food technique, the extracts of *Azadirachta indica* followed by *Eucalyptus citrodora* and *Prosopis julifera* were found to successfully inhibit the mycelial growth of the fungus. Sheep urine at 10% concentration, buffalo urine and goat urine at 20 per cent concentration recorded complete inhibition of *P. grisea*. Among the various bio inoculants *P. fluorescens* followed by *Serratia marcense* recorded a growth inhibition of 85.8 & 79.0% respectively.

Keywords **:** Rice, Blast, Plant extracts, Bio control agents, Animal products

Introduction

Rice (*Oryza sativa*) is considered as the most significant food crop for mankind because it feeds more than 50% of the world population (Zhang and Xie, 2014). India is the largest rice growing country accounting for about one third of the world acreage under the crop. In general, rice crop is subjected to attack by 50 diseases including 6 bacterial, 21 fungal, 4 nematode, 12 viral and 7 miscellaneous diseases and disorders (Jabeen *et al.,* 2012), among which rice blast caused by *Pyricularia oryzae* is considered as one of the most important diseases of rice (*Oryza sativa* L.) because of its wide distribution and destructiveness under favorable conditions resulting in serious loss of yield (Ou, 1985).

Chemical pesticides are reported to be highly effective in blast control (Yamaguchi, 2004) however, obvious pollution problems in the environment, and the toxic effects of synthetic chemicals on non-target organisms have prompted extensive searches for biofungicides that are environmentally safe and easily biodegradable (Gnanamanickam, 2002).

Bhardwaj *et al*. (2011) reported that many natural products, including plant extracts, have been shown to possess marked inhibitory activity against a variety of pathogens. Jaiganesh *et al*. (2007) reported that several bio inoculants produced chitinolytic enzyme which degraded fungal cell wall, induced of plant defence reaction and also produced certain antifungal low molecular weight molecules. With the above background, *in vitro* studies were carried out to evaluate the efficacy of certain plant extracts, natural products and bio agents against *Pyricularia grisea.*

Materials and Method

Preparation of Cold water extracts of plant leaves (Gerard Ezhilan *et al*., 1994).

Fresh leaves were washed with tap water and sterile water. It was then processed with sterile distilled water at 1 ml/g of tissue (1:1 V/W) with a mixie and filtered through a double layered cheese cloth. This formed the standard (100%) plant extract solution. The extracts were stored at 4°C temperature to avoid contamination and prospective chemical alteration.

Collection And Preparation Of Animal Excrement Extracts

Animal dung extracts (Sundarraj *et al.,* 1996)

Samples of animal dung (cow and goat) were collected from the floor of the animal house immediately after deposition. They were shade dried for one week and made into powder. The powdered animal litter was soaked in sterile distilled water @ 5 ml/g (5:1 V/W) and kept overnight, blended and filtered through a double layered cheese cloth. The filtrate thus collected was centrifuged at 10,000 rpm for 20 min. Then the supernatant solution was separated and added a few drops of toluene to avoid any bacterial contamination. This formed the standard (100%) animal excreta solution.

Animal urine (Raja and Kurucheve, 1997)

Freshly collected animal urine was added with a few drops of toluene to avoid any bacterial contamination and used as such forming the standard solution (100%). Animal urine was stored at 4°C when not in use.

Effect of plant products against *Pyricularia grisea* (Poisoned food technique, Dubey and Patel, 2000)

Extracts of six different plant products at 25 per cent concentration were incorporated with Oat Meal Agar (OMA) medium and poured into sterile Petri plates, allowed to cool and solidify. 5 mm mycelium disc of seven day old cultures of *P. grisea* were placed at the centre of the Petri plates and incubated at 25±2ºC for 10 days. The OMA without plant extracts served as control. Similarly, a synthetic fungicide Tricyclazole (75% WP) was tested against the fungi for comparison. For each treatment three replications were maintained.

Effect of plant extracts on spore germination of *P. grisea*

For each treatment, a concentration of 0.2% (v/v) spore suspension and plant extract was added to cavity slides, and were incubated at 25ºC. observed under microscopic fields at intervals of 8–10 h. A negative control treatment was

maintained using distilled water. The percent of spore germination was calculated by the following formula adopted by Dey *et al* (2013):

$$PG = \frac{A}{B} \times 100$$

Where: PG = Percent of spore germination, A = Number of spores germinated and B = Number of spores observed. Inhibition percent of spore germination was calculated using the formula by Vincet (1947).

$$\text{Inhibition } \% = \frac{C - T}{C} \times 100$$

Where: C = germination percent of spores in the negative control, T = germination percent of spores in the treatment.

***In vitro* testing of fungal antagonists** (Dennis and Webster, 1971)

The efficacy of different antagonistic organisms was tested by dual culture technique (Dennis and Webster, 1971) using PDA medium. The linear growth of antagonist and fungal pathogen / the type of interaction were observed on the fourth day after incubation.

***In vitro* testing of bacterial antagonists** (Yaqub & Shahzad, 2005)

The efficacy of *P. fluorescens, Bacillus* and *Serratia* collected at different places of Cuddalore District was tested against the pathogen by dual culture method in PDA. 8 mm disc of the pathogen was placed opposite to the bacterial streak. The plates were incubated at 25± 2°C. The linear growth of antagonist and pathogen / the type of interaction were observed on the third day after incubation.

The mechanism of antagonism was observed when the colonies of both fungi met. The following interactions between the test fungus and antagonist were noted (Yaqub & Shahzad, 2005).

A. Colonies of bio-control agent and test fungus met each other; the test fungus overgrew the colony of bio-control.
B. Growth of test fungus was inhibited by bio-control agent produced coiling around mycelium of the test fungus.
C. Colonies of bio-control agent and test fungus met each other, no further growth either of the test fungus or the bio-control agent was observed.
D. Colonies of the test fungus and bio-control agent intermingled.

Results and Discussion

Table 1 : Efficacy of various botanicals against the mycelial growth and spore germination of *Pyricularia grisea* (Poisoned Food Technique)

Botanical name	Conc. (%)	Colony diameter (mm)	% growth inhibition over control	Spore germination (%)	Germination inhibition (%)
Neem (*Azadirachta indica)*	25	32.5^{c}	63.8^{c} (53.0)	28.3^{e} (32.1)	69.2^{c} (56.3)
Henna *(Lawsonia inermis)*	25	18.9^{b}	79.0^{b} (62.7)	18.8^{f} (25.7)	79.5^{b} (63.1)
Yerukku *(Calotropis gigantean)*	25	64.1^{e}	28.7c (32.4)	47.8^{b} (43.74)	48.0^{e} (43.8)
Seemai karuvelam *(Prosopis julifera)*	25	72.9^{f}	19.0^{f} (25.8)	59.8^{c} (50.7)	35.0^{f} (36.3)
Arugampul *(Cynodon dactylon)*	25	61.3^{d}	31.8^{d} (34.32)	38.4^{d} (38.3)	58.2^{d} (49.7)
Eucalyptus *(Eucalyptus globulus)*	25	5.6^{a}	93.7^{a} (75.5)	8.9^{a} (70.6)	90.3^{a} (72.8)
Control		90.0	-	92.0	-

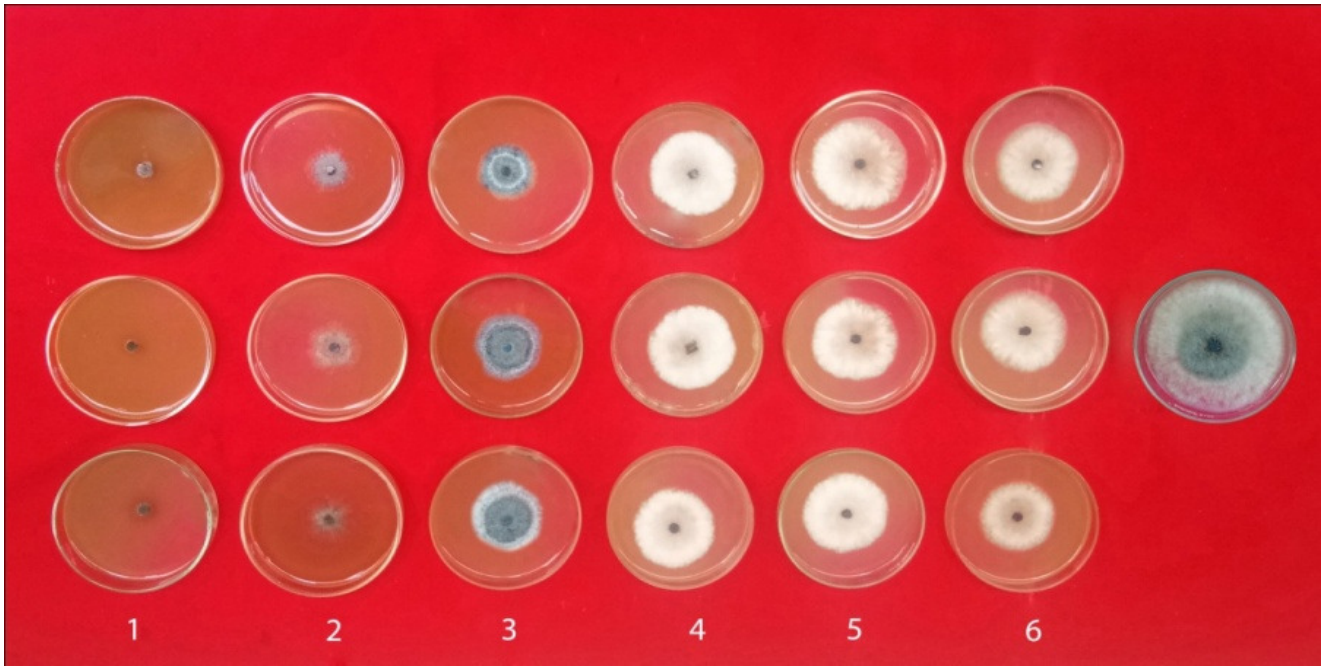
* Values are expressed as means ± S.D. for three replications in each group.
Values not sharing a common superscript differ significantly at P <0.05 (DMRT).

Evaluation of various botanicals against *P. grisea* Cav.

The results presented in table 1 revealed that all the plant extracts were found to be superior when compared to control in inhibiting the mycelial growth of *P. grisea*.

Among the phyto extracts, least mycelial growth of *P. grisea* was recorded with the extract of Eucalyptus with 5.6 mm and 93.7% growth inhibition over control, followed by Henna recording a radial growth and per cent growth inhibition of (18.9 mm & 79% respectively). Next best in order of merit were Neem (32.5 mm), Arugampul (61.3 mm), Yerukku (64.1 mm), Seemaikaruvelam (72.9mm). Similarly, Eucalyptus recorded the least per cent germination of spores (8.9%) followed by Henna recording a spore germination percentage of (18.8%) with a high inhibition percentage (79.5%).

Effectiveness of some botanicals like *Eucalyptus citrodora*, *Lantana camara, Ipomoea camara, Jasminum dispermum, Agave americana* (Ashok Kumar, 2006) has also been reported against *P. grisea*.. These finding lends support to the present work. This experiment proves that the plant extracts has potential antifungal properties against *P. grisea.*



Various botanicals (30%) against *P. oryzae* (Poisoned Food Technique)

1. Eucalyptus 30%+ OMA, 2. Neem 30%+ OMA, 3. Henna 30%+ OMA, 4.Arugampul 30%+ OMA, 5. Yerukku 30%+ OMA and 6. Seemaikaruvelam 30%+ OMA

Table 2 : Efficacy of certain Natural Products against the Mycelial Growth of *Pyricularia grisea*

Sl. No.	Sources	Diameter of mycelial growth (mm)					% Decrease over control				
		5%	10%	20%	30%	40%	5%	10%	20%	30%	40%
1	Sheep urine	1.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	0.0^{a}	98.8^{a} (83.7)	100^{a} (90.0)	100^{a} (90.0)	100^{a} (90.0)	100^{a} (90.0)
2	Goat urine	35.5^{b}	28.9^{c}	0.0^{a}	0.0^{a}	0.0^{a}	60.5^{b} (51.0)	67.8^{c} (55.4)	100^{a} (90.0)	100^{a} (90.0)	100^{a} (90.0)
3	Cow urine	45.3^{d}	33.8^{e}	15.4^{b}	0.0^{a}	0.0^{a}	49.6^{d} (44.8)	62.4^{e} (52.2)	82.8^{b} (65.5)	100^{a} (90.0)	100^{a} (90.0)
4	Buffalo urine	40.5^{c}	24.5^{b}	0.0^{a}	0.0^{a}	0.0^{a}	55.0^{c} (47.9)	72.7^{b} (58.5)	100^{a} (90.0)	100^{a} (90.0)	100^{a} (90.0)
5	Goat dung	59.6^{e}	32.7^{d}	25.7^{c}	21.6^{b}	14.8^{b}	33.7^{e} (35.5)	63.6^{d} (52.9)	71.4^{c} (57.7)	76.0^{b} (60.7)	83.5^{b} (66.0)
6	cow dung	60.2^{f}	41.9^{f}	32.6^{d}	25.4^{c}	21.5^{c}	33.1^{f} (35.1)	53.4^{f} (46.9)	63.7^{d} (52.9)	71.7^{c} (57.9)	76.1^{c} (60.7)
7	Tricyclazole @ 0.1%	0.0	-	-	-	-	100	100	100	100	100
8	Control	90.0	-	-	-	-	-	-	-	-	-

* 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).

EFFECT OF VARIOUS NATURAL PRODUCTS AGAINST *P. grisea*

The data from Table 2 revealed that sheep urine at 10% concentration recorded complete inhibition of *P. grisea*. Buffalo urine and goat urine recorded a 100 per cent decrease over control at 20 per cent concentration. The results clearly indicated that the animal dung required higher concentrations for exerting complete inhibition of the test pathogen. Goat dung and cow dung showed inhibition of mycelial growth but to a lesser extent recording an inhibition percentage of 83.5 and 76.1% respectively at 40 per cent concentration, whereas the standard chemical fungicide Triacyclozole at 0.1 % conc. recorded complete inhibition of the test pathogens.

Table 3 : Antagonistic effect of different bio agents against *P. grisea* (Dual culture method)

Sl. No	Test Organism	Av. Colony Diameter of Pathogen (mm)	% Growth Inhibition	Type of Interaction
1.	*Pseudomonas fluorescens*	7.2^{a}	85.8^{a} (67.9)	C
2.	*Bacillus subtilis*	15.1^{c}	70.2^{c} (56.9)	C
3.	*Trichoderma viride*	22.4^{e}	56.1^{e} (48.5)	D
4.	*Trichoderma harzianum*	21.8^{d}	57.2^{d} (49.1)	D
5	*Serretia marcense*	11.2^{b}	78.0^{b} (62.0)	C
6	Control	51^{f}	-	-

*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).

Antagonistic effect of different bio agents against *P. grisea* (Dual culture method).

The competitive ability of antagonists against *P. grisea* was studied by dual culture method. The results from Table 3, revealed that, there were significant differences among the different bio agents evaluated against *P. grisea,* and that maximum inhibition of the pathogen was observed in *Pseudomonas fluorescens*(85.8%) when compared to control followed by *Serretia marcense* (78.0%) and *Bacillus subtilis* (70.2%). As for the type of interaction between these colonies of bio-control agent and test fungus, (C) colonies of *Pseudomonas fluorescens, Bacillus subtilis* and *Serratia marcense* met *P. grisea*, no further growth of either the test fungus or the bio-control agent was observed. But when the colonies of *T. harzianum* and *T. viride* met with the colonies of *P. grisea* (D) the colonies of the test fungus and bio-control agents were intermingled.

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