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EFFICACY OF BIOFUNGICIDES ON THE YIELD PARAMETERS AND PDI OF RICE, CHALLENGE INOCULATED WITH *PYRICULARIA ORYZAE*, THE INCITANT OF BLAST DISEASE

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ABSTRACT

Among the eco-friendly products tested viz., leaf extracts of *eucalyptus* and henna @ (25% conc.), Sheep urine @ (10%), goat urine @ (20%) and *Pseudomonas fluorescens* @ (10g/kg of seed) alone and in combination, seeds treated with sheep urine was found to increase the germination percentage, vigour index and also enhance the yield parameters of rice. Among the different ecofriendly products, seeds treated with *P. fluorescens* retained the fungitoxicty for the longest duration of time against P. oryzae. Finally, under the pot culture studies, seed treatment with *P. fluorescens* and foliar spray with sheep urine on 30 and 50 DAT recorded the least leaf blast intensity in rice.

Keywords: Rice, blast, bio-fungicides, Animal products

INTRODUCTION

Rice (*Oryza sativa*), an important staple food crop and is considered as the most significant food crop for mankind because it feeds more than 50% of the world population (Zhang and Xie, 2014). In general, rice crop is subjected to attack by 50 diseases which includes 6 bacterial, 21 fungal, 4 nematode, 12 viral and 7 miscellaneous diseases and disorders (Jabeen *et al.,* 2012). Among the biotic stresses, rice blast is the most harmful fungal disease, which can lead to losses in rice yield up to 70 to 80% and is distributed worldwide, prevailing in more than 85 countries of the world (Gilbert *et al*., 2004).

Frequent use of fungicides on crops may cause hazards to human beings, plant health, beneficial micro-organisms, and develop fungicide resistance into the pathogens and residual toxicity in plant parts. Hence, it is vitally important to develop successful, eco-friendly, long lasting and economical solutions to crop loss caused by plant diseases. Therefore, extensive search for biofungicides that are environmentally safe and easily biodegradable have been carried out during the last two decades (Gnanamanickam, 2002).

This present study was proposed to find out efficacy of eco friendly products *viz*., extracts of eucalyptus leaf @ 25% conc., extracts of henna leaf @ (25% conc.), Sheep urine @ (10% conc.), goat urine @ (20% conc.) and talc formulation of *Pseudomonas fluorescens* @ (10g/kg of seed), alone and in combination against the blast disease incidence, growth and yield parameters of the host.

MATERIALS AND METHODS

Inoculum preparation (Xia *et al.*, 1993)

P. grisea cultures inoculated aseptically in maize bits substrate were incubated for 15 days at 28°C in the dark. Sporulating cultures were scraped with a metal spatula under sterile conditions and the mixture of conidia and mycelium was stirred for one minute in sterile distilled water. The resulting suspension was filtered through muslin to remove the mycelium. Spore concentration was adjusted with water containing 0.05% of Tween so as to give a final concentration of 10^5spores ml^{-1} (Xia *et al.*, 1993).

Seed treatment of rice seeds (wet seed treatment)

The rice variety TKM-9 susceptible to *Pyricularia grisea* was used for experiments. Seeds were soaked in 100 ml suspensions of different eco friendly products at different concentrations *Viz.*, eucalyptus and henna leaf extracts @ 25 per cent concentrations, sheep urine @ (10%), goat urine @ (20%), *Pseudomonas fluorescens* @ 10g/kg of rice seed and fungicide Tricyclazole (75% WP) @ 0.1 per cent concentration was used for comparison for 30 min. (Misra and Dharam *et al.,* 1990). Treated seeds were dried for 24 hours on sterile filter paper at room temperature then inoculated with the pathogen. Inoculated control seeds were first soaked in distilled water and then inoculated with the pathogens. Seeds soaked in distilled water served as untreated control.

Efficacy of seed treatment with eco-friendly products on the growth parameters of paddy challenge inoculated with *P. grisea* (Roll Towel Method) (ISTA, 1976)

The seeds treated with the different organic products were placed equidistantly between two moist sheets of germination paper towel (27 x 20 cm), rolled carefully ensuring no pressure on the seed, wrapped with polythene sheet to reduce surface evaporation and kept in germination chamber in an upright position and incubated at room

Table 1: Efficacy of seed treatment with eco-friendly products on the growth parameters of paddy challenge inoculated with *P. grisea.*

Tr. No	Treatments	Conc.(%)	Germination (%)	Shoot length (cm)	Root length (cm)	Vigour index
1	*Eucalyptus* leaf extract	25	92c	10.56e	11.04d	1857.6d
2	Henna leaf extract	25	90d	9.05f	10.69e	1677.9f
3	Sheep urine	10	94b	12.24b	12.45a	2172.7b
4	Goat urine	20	92c	11.78c	16.12b	2055.4c
5	*Pseudomonas fluorescens*	10g/kg of seed	92c	11.11d	10.09f	1823.2e
6	Tricyclazole	0.1	97a	12.42a	11.55c	2205.2a
7	Uninoculated control	-	90d	9.80g	10.02g	1684.7g
8	Inoculated control		88e	8.72h	8.64h	1266.3h

* 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).

Table 2: Retention of fungitoxicity of different products by seed treatment, challenge inoculated with P. grisea at different durations (Pot culture)

Tr. No	Treatments	Conc. (%)	PDI (%)20 DAI	PDI (%) 30 DAI	PDI (%) 40 DAI
1	Eucalyptus	25	08.7g	14.9f	28.4e
2	Henna	25	09.6h	16.8g	31.2g
3	Sheep urine	10	05.8c	10.7c	24.4d
4	Goat urine	20	06.9e	13.7e	29.9f
5	Pseudomonas fluorescens	10g/kg of seed	07.5f	12.5d	16.6c
6	Tricyclazole	0.1	00.0a	00.0a	03.9a
7	Uninoculated control	-	01.6b	04.7b	06.3b
8	Inoculated control	-	13.7h	37.6h	43.8h

DAT – Days after treatment
* 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).

Table 3. Effect of eco-friendly products on the Per cent disease incidence (PDI) of blast in paddy challenge inoculated with *P. grisea* (Pot culture)

Treatment	Treatments	Method of Application	Leaf blast intensity (%)	Neck blast intensity (%)
T1	*Eucalyptus*	ST	25.3i (30.1)	20.2i (26.7)
T2	Sheep urine	ST	23.6h (29.0)	18.5h (25.4)
T3	*P. fluorescens*	ST	23.0h (28.6)	17.9h (25.0)
T4	*Eucalyptus* (Foliar spray 30 and 50 DAT)	ST+FS	17.4f (24.6)	12.3e (20.5)
T5	Sheep urine (Foliar spray 30 and 50 DAT)	ST+FS	15.2e (22.9)	12.7f (20.8)
T6	*P. fluorescens* (Foliar spray 30 and 50 DAT)	ST+FS	14.2d (22.1)	10.1d (18.5)
T7	*P. fluorescens* (ST) + Eucalyptus (FS - 30 and 50 DAT)	ST+FS	11.4c (19.4)	9.8c (18.2)
T8	*P. fluorescens* (ST) + Sheep urine (FS - 30 and 50 DAT)	ST+FS	09.2a (17.5)	8.4b (16.8)
T9	Tricyclazole @ 0.1 %	ST+FS	08.7b (17.0)	7.3a (15.6)
	Inoculated control		58.6k (49.9)	48.2k (43.9)
	Uninoculated Control		12.6g (20.6)	13.4g (20.8)

* 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).

temperature 28±2°C for fifteen days. Each treatment was replicated thrice. Shoot length, root length and vigour index were calculated using the standard procedures after the incubation period is over.

Germination percentage was recorded on the 7th day by using the formula,

Root length and shoot length

Ten representative normal seedlings from the Roll towel method were taken from each replication. The shoot length

was measured from the collar to the tip of the leaf and root length from the collar region to the largest primary root and the respective mean values were recorded in cm.

Seedling Growth and vigour

The vigour index (V_1) was calculated by using the formula suggested by Abdul Baki and Anderson (1973). V_1 = (Root length + Shoot length) x Germination percentage.

Retention of fungitoxicity of different products by seed treatments

The seeds of rice variety TKM-9 were soaked and stirred in 100 ml suspensions of different eco friendly products as in the treatment schedule at different concentrations for 30 min (Dharam *et al.,* 1971). Treated seeds were dried for 24 hours on sterile filter paper and then inoculated with spore suspensions of *P. grisea*. The per cent disease index (PDI) was calculated on the 20th , 30th and 40th days of treatment (DAT).

Leaf blast incidence (Pot Culture Experiment)

Experiments were conducted in the experimental farm Department of the Plant Pathology, Annamalai University. Blast susceptible variety TKM-9 was chosen for the experiment. Each treatment was replicated thrice in a randomized block design. The seeds inoculated with the pathogen were treated with different eco-friendly products and sown in pots. The transplanted plants were sprayed with eco-friendly products on the 30th and 50th DAT. Incidence of blast disease was recorded before harvesting based on the Standard Evaluation System adopting 0-9 grade system (SES, 1996).

The per cent disease index (PDI) was calculated by adopting the formula:

RESULTS AND DISCUSSION

Efficacy of seed treatment with eco-friendly products on the growth parameters of paddy challenge inoculated with *P. grisea*.

Among the biological inputs, sheep urine at 10% conc. showed increased germination percentage (94%) followed by eucalyptus leaf extract @ 25%, goat urine @ 25% and *Pseudomonas fluorescens* @ 10g/kg of seed which were on par with each other recording a germination percentage of (92%) each. Sheep urine followed by goat urine recorded the maximum shoot length, root length and vigour index (12.24 cm, 12.45 cm & 2172.7 and 11.78 cm, 12.12 cm & 2055.4) respectively. Least vigour index was recorded by inoculated control (1266.3). Standard fungicide Tricyclazole (Beam 75 WP) was found to be superior to all other treatments tested.

Discussion

Sivaprakash and Kurucheve (1999) observed stimulatory effect of seedling growth and vigour of rice seedlings by the mixture of *Allium sativum* + Eucalyptus leaf extracts and pig dung whereas in tomato seedlings, mixture of garlic and *Lawsonia* extract exhibited maximum vigour. The enhancement of seed germination root length and shoot length by the plant extracts might be due to several factors such as the presence of auxins in the plant extract and also by the presence of hormone synthesizing organisms as phyllosphere population. The fungitoxic principle in the plant products may kill the pathogens present both internally and externally in the seeds. The mechanisms of plant growth promotion by PGPR include production of plant growth regulators like auxins, gibberellins and cytokinins (Dubeikovsky *et al.,* 1993).

Retention of fungitoxicity of different products by seed treatment, challenge inoculated with *P. grisea* (Pot culture)

Experiments were conducted to evaluate the duration of retention of fungitoxicity of different eco-friendly products against *P. grisea*. The data from the Table 2 shows that on the 20th and 30th DAT Sheep urine, goat urine and *Pseudomonas fluorescens* recorded a PDI of (5.8% & 10.7%), (6.9% & 13.7%) and (7.5% & 12.5%) when compared to control with (13.7 & 37.6%) PDI respectively.

On the 40th day standard test fungicide Tricyclazole (Beam 75 WP) was successful in reducing PDI of blast disease up to 40 days (3.9%) which was very significant when compared to inoculated control with a PDI of (43.8%). On the 40th DAT next best in order of merit was *Pseudomonas fluorescens* (16.6%) followed by sheep urine and eucalyptus leaf extracts recording a PDI of 24.4% and 28.4% respectively which revealed that the fungitoxic effect of the organic products especially animal excrements and plant extracts showed severe reduction after 30 days of treatment.

Effect of eco-friendly products on the Per cent disease incidence (PDI) of blast in paddy, challenge inoculated with *P. grisea* (Pot culture).

Inoculated control recorded a severe blast incidence of 58.6% and an uninoculated check showed an incidence of 12.6%. All the eco-friendly products were found to significantly reduce the blast incidence when compared to control. The results revealed that among the different products tried seed treatment with *P. fluorescens* and sheep urine were on par with each other recording a leaf blast intensity of 23.0 & 23.6 per cent followed by extracts of eucalyptus (25.3%). Whereas application of *P. fluorescens* by seed treatment and foliar spray (20 & 40 DAT) reduced the disease intensity to 14.2% followed by sheep urine (15.2%) and extracts of eucalyptus (17.4%). However, seed treatment with *P. fluorescens* in combination with foliar spray with sheep urine showed a further decrease in blast incidence recording 9.2 per cent leaf blast and 8.4% neck blast followed by seed treatment with *P. fluorescens* and foliar spraying with extracts of eucalyptus also showed significant reduction of the leaf blast and neck blast (11.4 & 9.8%) respectively. Tricyclazole (0.1%) recorded the

least leaf and neck blast disease incidence when compared to control.

Discussion:

The effect of plant products might be due to the fungicidal nature which inhibits the disease and the presence of growth promoting substances which enhances the growth and yield parameters of the host. There are three major nitrogenous compounds excreted by animal's *viz*., ammonia, urea and uric acid (Wright, 1995), which has the capacity to kill plant-pathogenic fungi and influence the growth of plants. Similar, work carried out by Gnanamanickam *et al*. (1990) found that *Pseudomonas fluorescens* strain was most effective against *P. grisea* Cav. as it produced fluorescent antiblast antibiotics in culture which inhibited the conidial germination of *P. grisea* cav. *in vitro*. Sy *et al.* (1990) found maximum inhibition of growth and conidial germination of *P. grisea* by *Trichoderma harzianum* and attributed it to the pronounced colonization by antagonists in advance to the pathogens. Antifungal properties of plant products against *P. grisea* the rice blast pathogen has also been reported by Kamalakannan *et al*. (2001). The fungitoxic properties of animal excreta against different pathogens in liquid medium have already been reported by several workers (Rajendraprasad and Kurucheve, 2002).

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