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Ecofriendly approaches for the management of grain discolouration in rice

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ABSTRACT: Ecofriendly methods for the management of grain discolouration in rice using plant oils, plant extracts and bacterial antagonist were tested in pot culture and field experiments. The field fungi such as *Drechslera oryzae, Curvularia lunata* and *Fusarium moniliforme* were predominantly associated with the grain discolouration in rice. In pot culture experiment, post inoculation spraying of neem oil 80 EC (3%) was highly effective in reducing the grain discolouration which was on par with carbendazim (250 g ha^{-1}), rhizome extract (10%) of *Curcuma longa*, leaf extract (10%) of *Nerium oleander*, *Pseudomonas fluorescens* (Pf1) (10^{9} cfu ml^{-1}) and leaf extract (10%) of *Vinca rosea.* In the field, spraying of neem oil 80EC (3%) at flowering stage and ten days later reduced the grain discolouration from 21.60 to 11.45 per cent which was on par with carbendazim (250 g ha^{-1}), rhizome extract (10%) of *C. longa*, leaf extract (10%) of *N. oleander* and *P. fluorescens* (Pf1) (10^{9}cfu ml^{-1}). There was also significant increase in the grain yield due to these treatments compared to control.

KEY WORDS: Field fungi, grain discolouration, plant extracts, plant oils, *Pseudomonas fluorescens.*

INTRODUCTION

Rice yield and quality are severely affected by pests and diseases. Grain discolouration is recognized as one of the major diseases in the maximization of rice production (Malavolta and Bedendo, 1999). Field and storage fungi cause grain discolouration in rice. Rice is mostly grown in the wet season which is congenial for infection of seed with a number of fungi (Pandey *et al.,* 2000). The fungal association with discoloured seeds results in deterioration of nutritional value of seeds due to physical, physiological and biochemical changes in the seeds (Narain, 1992). Grain discolouration poses a serious problem in seed certification. It also reduces the commercial value of seeds due to their appearance and change in nutritional value (Ray and Gangopadhyay, 1991). In Tamil Nadu, two fungi *viz., Drechslera oryzae* and *Curvularia lunata* were found to be predominantly associated with discoloured rice grains (Subramanian, 1988). Field fungi such as *D. oryzae*, *C. lunata, Sarocladium oryzae, Trichoconiella padwickii, Fusarium moniliforme, Nigrospora* spp., *Pyricularia grisea* and *Microdochium oryzae* were predominantly associated with grain discolouration according to several workers (Vaid and Sharma, 1992; Saifulla *et al*., 1996). None of the fungicides reported so far provide complete control of seed discolouration (Misra and Dharma Vir, 1992). Moreover continuous use of chemicals leads to the development of fungicide tolerant strains of pathogens. Now a days many biocontrol agents, plant extracts and plant oils are being used for the control of various plant pathogens. The pests and pathogens do not develop resistance to them because it requires several simultaneous mutations to occur in the genetic constituents of pests and pathogens to overcome the numerous ingredients of botanical fungicide (Das and Das, 1994).

MATERIALS AND METHODS

Isolation of pathogens

Pathogenic fungi associated with the rice grains were isolated by following the Blotter method and Agar plate method (Agarwal and Sinclair, 1993).

Blotter method

Association of sporulating fungi in the discoloured grains was assessed by the blotter method. The seeds were placed on three layers of filter paper moistened with sterile water on sterile Petri plates. The plates were incubated by exposing them to alternate cycles of NUV (near ultra violet) light and darkness for 12 h duration at room temperature (28 ± 2ºC) for seven days. Four hundred seeds were examined under stereobinocular microscope. The pathogens were identified by preparing slides and examining them under compound microscope (Mew and Misra, 1994). The fungi were purified by single spore

isolation method (Riker and Riker, 1936), subcultured and maintained on potato dextrose agar slants.

Agar plate method

Surface sterilized, discoloured rice grains were plated on sterile Petri plates containing 20 ml of potato dextrose agar medium. Ten grains were plated on each Petri dish and incubated at 22ºC for 5 - 8 days either under alternate cycles of NUV light and darkness or in darkness (ISTA, 1976).

Pathogenicity of the fungal isolates

The pathogenicity of the isolated fungi *viz., D. oryzae*, *F. moniliforme,* and *C. lunata* were tested separately and in combination on the rice variety MDU 5 grown in pots. Spore suspension (10^7 spores ml^{-1}) of the pathogens were sprayed on the panicle at flowering, milky, soft-dough and dough stages. Suitable control (sterile water spray) was also maintained. The panicles were covered with polythene bags for two days. Five replications were maintained for each treatment. The percentage of grain discolouration was worked out by counting the healthy and discoloured grains in ten earheads.

Preparation of plant extracts

Plant extracts were prepared as described by Shekhawat and Prasada (1971). The fresh leaves/plant parts collected were ground with sterile water 1 ml g^{-1} in a pestle and mortar. The extracts were filtered through muslin cloth and finally through Whatman No. 1 filter paper. The extract was passed through Seitz filter to free the extract of the bacterial contaminants. This formed the standard plant extract solution (100%). The extract was further diluted to ten per cent concentration using sterile medium.

***In vitro* efficacy of antagonists**

The antagonistic effect of *P. fluorescens* (Pf1) and *Bacillus subtilis* were tested against the three pathogenic fungi by dual plate method (Dennis and Webster, 1971). A nine mm PDA culture disc of the pathogen was cut individually from seven day old culture and placed at one side on the sterilized PDA plates. Simultaneously, the actively growing *P. fluorescens* (Pf1) and *B. subtilis* cultures were separately streaked on the opposite side. Four replications of each treatment and suitable controls were maintained. The plates were incubated at room temperature (28 ± 2ºC) for seven days. The mean diameter of the mycelial growth was measured and the results were expressed in terms of per cent inhibition of the mycelium over control (Vincent, 1927).

The antagonistic effect of *Trichoderma harzianum*, *T. viride* and *T. reesei* were tested against the isolated seed borne pathogens viz., *D. oryzae, F. moniliforme* and *C. lunata* by dual plate technique (Dennis and Webster, 1971). Four replications of each treatment and suitable controls were maintained and the results were expressed in terms of per cent inhibition of mycelium over control (Vincent, 1927).

Effect of *P. fluorescens* and plant products on rice grain discolouration in pot culture experiment (artificial inoculation)

For the management of grain discolouration in rice, the effect of *P. fluorescens* and plant products on rice grain discolouration was studied in pot culture experiment with artificial inoculation of the pathogens. Twenty one day old MDU5 rice seedlings were transplanted in 30 cm dia pots separately. Five seedlings were maintained for each treatment. Each treatment was replicated thrice. Carbendazim (0.1%) was used as standard check. MDU5 rice plants were sprayed with the spore suspension (10^7 spores ml^{-1}) of *D. oryzae, F. moniliforme* and *C. lunata* at flowering stage of the crop. This was followed by spraying of treatments 72 h after spray inoculation of the pathogen. A second round of treatment was given 10 days later. Both inoculated and uninoculated controls were maintained. The percentage of grain discolouration was worked out by counting the healthy and discoloured grains in ten earheads.

Effect of *P. fluorescens* and plant products on rice grain discolouration in the field

A field trial in a randomized block design with 12 treatments and three replications was conducted during rabi season of 2005-2006 at Agricultural college and Research Institute, Madurai to evaluate the efficacy of *P. fluorescens*, plant extracts and plant oils. Twenty one day old MDU5 rice seedlings were transplanted adopting the recommended spacing (12.5 x 10 cm) and the fertilizer dosage (NPK: 120-38-38 kg ha^{-1}). The first spray was given at flowering stage and the second spray was given ten days later. The numbers of healthy and discoloured grains were counted for each treatment by selecting ten ear heads at random and the percentage of grain discolouration was calculated. The grain yield was recorded at the time of harvest. The treatments were the same as in pot culture experiment.

RESULTS AND DISCUSSION

The pathogens *D. oryzae*, *F. moniliforme* and *C. lunata* when artificially inoculated individually on rice panicles were able to cause 77.39, 67.44 and 74.03 per cent infection respectively while the mixed inoculum of these three pathogens caused the highest infection (81.80%). The artificial inoculation caused maximum infection during flowering stage and minimum during dough stage (Table 1). Pandey *et al*. (2000) reported that *Gibberella fujikuroi* exhibited light brown discolouration and ashy gray with

Table 1. Pathogenicity of the fungal isolates (artificial inoculation)

Stages of earhead	Per cent infection*					
	D. oryzae	*F. moniliforme*	*C. lunata*	*D. oryzae* + *F. moniliforme* + *C. lunata*	Control	Mean
Flowering	95.60 (78.12)	89.50 (71.14)	92.75 (74.49)	97.20 (80.71)	0.00 (12.92)	93.76 (62.18)
Milky	92.20 (73.88)	79.25 (62.92)	89.73 (71.41)	94.40 (76.48)	0.00 (12.92)	88.90 (58.22)
Soft-dough	83.50 (66.11)	65.50 (54.04)	73.65 (59.13)	85.32 (67.51)	0.00 (12.92)	76.99 (50.64)
Dough	38.24 (38.20)	35.50 (36.57)	40.00 (39.23)	50.27 (45.16)	0.00 (12.92)	41.00 (33.11)
Mean	77.39 (64.08)	67.44 (61.06)	74.03 (56.16)	81.80 (67.47)	0.00 (12.92)	75.17 (51.04)
	CD (P = 0.05)					
Stages	0.96					
Pathogens	1.08					
S x P	2.15					

Mean of five replications; data in parentheses are arcsine transformed values

Table 2. *In vitro* efficacy of antagonists against field fungi

Antagonists	*Mycelial growth (cm) of *D. oryzae*	Per cent growth inhibition	*Mycelial growth (cm) of *F. moniliforme*	Per cent growth inhibition	*Mycelial growth (cm) of *C. lunata*	Per cent growth inhibition
B. subtilis	3.50	60.67	5.20	41.57	3.00	66.29
P. fluorescens	4.20	52.81	2.83	68.20	4.00	55.06
T. viride	2.44	72.58	4.12	53.71	2.65	70.22
T. harzianum	2.95	66.85	3.57	59.89	4.85	45.51
T. reesei	2.76	68.99	5.10	42.70	4.46	49.89
Control	8.90	-	8.90	-	8.90	-
CD (P = 0.05)	0.69		0.60		0.86	

*Mean of four replications

black dots in seed coat and endosperm. According to Rathaiah (1997) grain discolouration due to *D. oryzae* was predominant during soft dough stage of rice. Our results support the findings Subramanian (1988) who also found that flowering stage was the most susceptible stage for grain discolouration in rice .

***In vitro* efficacy of antagonists**

P. fluorescens (Pf1) was the most effective bioagent against *F. moniliforme* with maximum inhibition of mycelial growth (68.20%) was recorded. Next best was *T. harzianum* followed by *T. viride* and *T. reesei*. While considering the efficacy of antagonists against *D. oryzae*, *T. viride* was on par with *T. reesei* and *T. harzianum. T. viride* was on par with *B. subtilis* which recorded 70.22 and 66.29 per cent growth inhibition of *C. lunata*. *P. fluorescens* (Pf1), *T. reesei* and *T. harzianum* were on par in the inhibition of mycelial growth of *C. lunata* (Table 2).

Effect of *P. fluorescens* and plant products on rice grain discolouration

Pot culture experiment

The results of the pot culture experiment revealed that spraying of neem oil 80 EC (3%) at flowering stage and at 10 days later was the best in inhibiting the grain discolouration (45.78% disease reduction) and was on par with carbendazim (250g ha^{-1}), rhizome extract (10%) of *C. longa*, leaf extract (10%) of *N. oleander, P. fluorescens* (Pf1) (10^9 cfu ml^{-1}) and leaf extract (10%) of *V. rosea*. These were followed by pungam oil 80EC and palmarosa oil

Table 3. Effect of *P. fluorescens* and plant products on rice grain discolouration in pot culture (artificial inoculation)

Treatments	*Per cent grain discolouration	Disease reduction (%)
Palmarosa oil 80EC (0.1%)	21.64 (27.71)	34.82
Neem oil 80EC (3%)	18.00 (25.06)	45.78
Pungam oil 80EC (3%)	20.80 (27.12)	37.35
Nerium oleander leaf extract (10 %)	19.50 (26.16)	41.27
Pithecolobium dulce leaf extract (10 %)	29.50 (32.88)	11.14
Vinca rosea leaf extract (10 %)	20.20 (26.69)	39.16
Ocimum sanctum leaf extract (10 %)	26.50 (30.97)	20.18
Curcuma longa rhizome extract (10 %)	19.00 (25.82)	42.77
Pseudomonas fluorescens (Pf1) talc based (10^9 cfu ml^{-1})	20.00 (26.55)	39.76
Seedling dip with *Pseudomonas fluorescens* (Pf1) (2.5Kg ha^{-1})	25.00 (29.99)	24.70
Carbendazim (250g ha^{-1})	17.50 (24.68)	47.29
Control (inoculated)	33.20 (35.17)	-
Control (Uninoculated)	0.00 (12.92)	-
CD (P = 0.05)	2.79	

Mean of three replications; data in parentheses are arcsine transformed values

Table 4. Effect of *P. fluorescens* and plant products on rice grain discolouration in the field

Treatments	*Per cent discolouration	Disease reduction (%)	*Grain yield (Kg ha^{-1})
Palmarosa oil 80EC (0.1%)	14.64 (22.45)	32.22	2150.00
Neem oil 80EC (3%)	11.45 (19.76)	47.00	2896.67
Pungam oil 80EC (3%)	14.01 (21.97)	35.14	2595.75
Nerium oleander leaf extract (10 %)	12.69 (20.86)	41.25	2610.50
Pithecolobium dulce leaf extract (10 %)	17.83 (24.98)	17.45	2050.63
Vinca rosea leaf extract (10%)	13.79 (21.79)	36.16	2510.00
Ocimum sanctum leaf extract (10%)	15.30 (23.02)	29.17	2083.33
Curcuma longa rhizome extract (10%)	12.60 (20.79)	41.67	2650.00
Pseudomonas fluorescens (Pf1) talc based (10^9 cfu ml^{-1})	13.32 (21.41)	38.33	2585.30
Seedling dip with *Pseudomonas fluorescens* (Pf1) (2.5kg ha^{-1})	14.71 (22.53)	31.89	2535.32
Carbendazim (250g ha^{-1})	11.24 (19.49)	47.96	3316.67
Control	21.60 (27.68)	0	2000.00
CD (P = 0.05)	1.93		331.78

*Mean of three replications

80EC (0.1%) which was on par with each other (Table 3). Leaf extract (10%) of *Pithecolobium dulce* was the least effective against grain discolouration.

Effect of *P. fluorescens* and plant products on rice grain discolouration in the field

Two sprays of neem oil 80EC (3%) given on rice plants (MDU 5) first at the flowering stage and second at 10 days later significantly reduced the incidence of grain discolouration. The incidence of grain discolouration in neem oil sprayed plot was 11.45 per cent as against 21.60 per cent in the control (Table 4). This was also on par with Carbendazim (250 g ha^{-1}), rhizome extract (10 %) of *C. longa*, leaf extract (10 %) of *N. oleander* and *P. fluorescens* (Pf1) (10^9 cfu ml^{-1}).

This result is also in line with the finding of Gangopadhyay (1998) who reported that turmeric application in aqueous $NaHCO_3$ (10 g/lit) was effective in reducing the incidence of *D. oryzae*, *Rhizoctonia solani*, *S. oryzae*, *P. grisea* and *C. lunata* in rice seeds. Nayar and Vidhyasekaran (1998) found that foliar spray of *P. fluorescens* strain P1 at 0.1 % (3.6 X 10^9 cfu L^{-1}) effectively inhibited the occurrence of pathogens *viz.*, *P. oryzae*, *D. oryzae* and *R. solani*. Fluorescent pseudomonads are also known to survive well in phyllosphere (Wilson *et al.,* 1992). Colonization of plant roots by *Pseudomonas* sp. also induces resistance against foliar pathogens (Liu *et al*., 1995). Induced systemic resistance (ISR) might be another mechanism for achieving biological control of plant diseases by fluorescent pseudomonads (Van Loon *et al.,* 1998). The yield was the highest *viz.,* (3316.67 kg ha^{-1}) in the carbendazim sprayed plots. Neem oil 80EC (3%) recorded the higher yield of 2896.67 kg ha^{-1} as against 2000kg ha^{-1} in the control. The plots sprayed with leaf extract (10%) of *Pithecolobium dulce* recorded the minimum yield (2050.63 kg ha^{-1}).

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