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RESEARCH PAPER

Eco-friendly management of rice sheath rot disease by phylloplane microflora

I. YESU RAJA* **AND** P. MAHALAKSHMI
Department of Plant Pathology, Agricultural College and Research Institute, MADURAI (T.N.) INDIA

Abstract : The micro-organisms isolated from the phylloplane of rice were *Aspergillus flavus, Aspergillus niger, Aspergillus glaucus, Penicillium* sp., *Curvularia* sp., *Cladosporium* sp., *Pseudomonas fluorescens* and *Bacillus subtilis.* Among these, *P. fluorescens* exhibited the maximum inhibition on the mycelial growth and sporulation of *S. oryzae* followed by *B. subtilis, A. flavus, A. niger* and *Cladosporium* sp. In pot culture, spraying of *P. fluorescens* (10^9cfu/ml) was the most effective in reducing the disease intensity by 68.56 per cent followed by *B. subtilis* (10^9 cfu/ml), *A. flavus* ($2x10^4$ spores/ml), *A. niger* (2×10^4 spores/ml) and *Cladosporium* sp. ($2x10^4$ spores/ml) which recorded 63.07, 44.42, 42.23 and 40.40 per cent disease reduction, respectively.

Key Words : Eco-friendly management, Phylloplane microflora, Rice sheath rot

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INTRODUCTION

Sheath rot caused by *Sarocladium oryzae* (Sawada) W.Gams and D. Hawksw has become endemic and one of the major constraints in production and off-setting the efforts to attain targetted levels of rice production in Tamil Nadu and also in other rice growing states. The average reduction in grain yield due to this disease was estimated to be 57.40 per cent in Tamil Nadu (Mohan, 1976).The maximum of 85 per cent yield loss was reported in Andhra Pradesh by Muralidharan and Venkata Rao (1980).

Disease management schedule requires effective integration of both short and long term strategies. As such no dependable sources of genetical resistance to sheath rot disease is available for exploitation. Some bacterial antagonists had been reported to be effective in controlling this disease (Sakthivel and Gnanamanickam, 1987).

MATERIAL AND METHODS

Isolation of phylloplance microflora of rice :

The actively growing fresh leaves were collected from 75- day- old CO 43 rice plants and cut into small bits by means of a sterile scalpel. The leaf bits were suspended in 10 ml of sterile distilled water and thoroughly shaken for five min and allowed to stand for five min. One ml of this suspension was pipetted out into each sterilized Petri plate using a sterile pipette. Fifteen ml of Martin's rose bengal agar medium (Martin, 1950) for isolation of fungi, King's B medium (King *et al.*, 1954) and nutrient agar medium (Allen, 1953) for bacteria and Kuster's agar medium (Kuster and Williams, 1964) for actinomycetes were separately poured into each of these plates, gently swirled for uniform suspension and allowed to solidify. Four replications were maintained. Sterile water inoculated plates served as control. The plates were incubated at room temperature (28 ± 2^0C). Forty eight h after incubation, the bacterial colonies were subcultured and subsequently purified by streak plate method (Rangaswami and Soumini Rajagopalan, 1973). The fungal colonies were subcultured after three to five days and purified by single hyphal tip method (Rangaswami, 1972). The growth of actinomycetes colonies was subcultured after 10 days. The isolated bacterial and fungal

*** Author for correspondence**

cultures were maintained on nutrient agar and PDA slants, respectively.

Identification of phylloplane fungi :

The isolated fungal cultures were identified by observing the mycelial and the conidial characteristics under the microscope.

Identification of phylloplane bacteria :

Pseudomonas fluorescens :

P. fluorescens was identified by the colony characters, gram staining, growth at 4^0C and fluorescence test.

Gram staining :

A dried smear was prepared on a glass slide and flooded with crystal violet staining reagent [Solution A: Crystal violet-2g and ethanol (95%) - 20ml. Solution B: Ammonium oxalate - 0.8g and distilled water - 80ml. Solutions A and B were mixed] for one min. The smear was washed in a gentle and direct stream of tap water for two sec. The smear was flooded with iodine solution (Iodine - 1g, potassium iodide - 2g and distilled water -300ml) for one min and the slide was washed in a gentle and direct stream of tap water for 2 sec. The smear was blotted to dry with absorbent paper and it was immersed in 95% (v/v) ethanol for 30 sec with gentle agitation. The smear was blotted to dry with absorbent paper and it was immersed in counter stain safranin [Safranin O (2.5% solution in 95% ethanol] -10ml and distilled water - 100ml] for 10 sec. It was washed in tap water and blotted to dry with absorbent paper. The slide was examined under the microscope. The bacterial cells were red colour (Gram negative) /blue colour (Gram positive) (Hucker and Conn, 1923).

Growth at 4^0C :

A loopful of bacterium was streaked from bottom to top in the slants contanining King's B medium under aseptic condition, incubated at 4^0C for three days and observed for growth (Laskin and Lechevalier, 1977).

Fluorescence test :

A loopful of the bacterial culture was streaked horizontally at the centre of Petri plates containing King's B medium under aseptic conditions, incubated at room temperature for 48h and observed for the production of pigments that showed fluorescence under ultra violet radiation of short wave length (Ca.254nm) (Laskin and Lechevalier, 1977).

Bacillus subtilis :

The bacterium *B. subtilis* was identified by the colony characters, gram staining and endospore staining.

Gram staining :

Gram staining was done as per the procedure already described (Hucker and Conn, 1923). The bacterial cells were blue colur (positive reaction).

Endospore staining :

A dired smear was prepared on a glass slide and covered with a small pieces of blotting paper. The blotting paper was saturated with freshly filtered Ziehl's carbol fuchsin [Solution A : Basic fuchsin (90% dye content) - 0.3g and ethanol (95%) - 10ml. Solution B : Phenol - 5g and distilled water - 95ml. Solutions A and B were mixed].Allowed to steam 5-10 min, keeping paper moist by adding more staining fluid and washed with tap water. A drop of saturated aqueous nigrosin (Nigrosin-10g and distilled water-100ml) was applied and spread evenly. The slide was allowed to dry with gentle heat without prior washing. The spores were red and the vegetative cells were unstained (Snyder, 1934).

Efficacy of rice phylloplane microflora against rice sheath rot pathogen *in vitro* :

The antagonistic activity of the phylloplane fungi and bacteria isolated from rice was assessed by dual culture technique (Dennis and Webster, 1971). A nine mm actively growing culture disc of the pathogen was placed onto the sterilized PDA medium previously poured into sterilized Petri plate approximately at a distance of 1.5 cm away from the periphery of the plate. Similarly a nine mm culture disc of the purified phylloplane test fungi (*Aspergillus flavus, A. niger, A. glaucus, Penicillium* sp., *Curvularia* sp. and *Cladosporium* sp.) was placed onto the medium at the opposite side of the plate. In the case of phylloplane bacteria, the pathogen *S. oryzae* alone was similarly inoculated onto PDA medium and incubated at room temperature (28 ± 2^0C) for three days. Then the actively growing 48 h old culture of the respective test bacteria *viz., P. fluorescens* and *B. subtilis* was separately streaked onto the medium at the opposite side of the plate at 1.5 cm away from the periphery of the Petri dish. Three replications were maintained for each treatment. The medium inoculated with the pathogen alone served as control. The plates were incubated at room temperature (28 ± 2^0C). The radial growth of the pathogen was measured after 15 days in all the treatments. The results were expressed as per cent inhibition of the mycelial growth of the pathogen over control.

To assess the effect on sporulation, four fungal culture discs from the growth of the pathogen in the above treatments were taken from different areas in the plate and placed in 20 ml of sterile distilled water in 100 ml flasks. The flasks were kept in a shaker for 15 min and the number of spores per ml of water was determined using haemocytometer. The per cent reduction in sporulation caused by the phylloplane microflora was calculated.

Efficacy of rice phylloplane microflora against sheath rot disease in pot culture :

The phylloplane microflora of rice *viz., A. flavus, A. niger,*

A. glaucus, Penicillium sp., *Curvularia* sp., *Cladosporium* sp., *P. fluorescens* and *B. subtilis* were evaluated for their efficacy against sheath rot disease in pot culture by artificial inoculation of the pathogen.

Spore suspensions of the phylloplane fungi were prepared from highly sporulating seven day old PDA cultures by superficially scrapping with a sterilized inoculation needle and then flooding with a nutrient solution containing two per cent sucrose, 0.1 per cent yeast extract and 0.5 per cent tween 80. Masses of agar and large mycelial fragments were removed by filtering through muslin cloth. Spore concentration was adjusted to $2x10^4$ spores per ml (Bansal *et al.*, 1988).

Two day old actively growing cells of *P. fluorescens* and *B. subtilis* were harvested from Petri plates culture, respectively containing King's B medium and nutrient agar medium by adding sterile distilled water and by gently disturbing the growth of the bacteria on the surface of the medium with a fine sterile brush. The bacterial suspension was collected and a bacterial load of 10^9 cfu per ml was obtained by adjusting the OD value of the suspension to 0.45 in a colorimeter.

Eighty five day old CO 43 rice plants grown in earthen pots and maintained in the glass house were sprayed with the fungal spore and bacterial cell suspensions of the phylloplane microflora as pre-inoculation spray. Another set of plants sprayed with distilled water served as control. After 24 h, the plants were inoculated using single grain culture of *S.oryzae* as already described. Ten days later, the second spraying was given with the same treatments on the respective sets of rice plants. Three replications were maintained. At grain maturity stage, the disease intensity was assessed based on the new scoring system developed by Narayanasamy and Viswanathan (1990). From this, per cent disease reduction for each treatment was calculated.

RESULTS AND DISCUSSION

Six fungi *viz.*, *Aspergillus flavus* Link., *A. niger* Van Tiegh., *A. glaucus* Link., *Penicillium* sp. Link. ex Fr., *Curvularia* sp. Boedijn, *Cladosporium* sp. Link. ex Fr. and two bacteria *viz.*, *Pseudomonas fluorescens* Migula and *Bacillus subtilis* (Ehrenberg) Cohn were isolated from rice phylloplane.

Table 1 : Efficacy of rice phylloplane microflora against mycelial growth of *Sarocladium oryzae*

Sr. No.	Phylloplane microflora	Mycelial growth (cm)*	Per cent growth inhibition*
1.	*Aspergillus flavus*	2.97	60.63 (51.14) [b]
2.	*Aspergillus niger*	3.47	54.01 (47.30) [c]
3.	*Aspergillus glaucus*	3.83	49.11 (44.49) [d]
4.	*Penicillium* sp.	5.47	27.48 (31.55) [f]
5.	*Curvularia* sp.	4.13	45.13 (42.20) [e]
6.	*Cladosporium* sp.	3.57	52.65 (46.52) [cd]
7.	*Pseudomonas fluorescence*	1.17	84.51 (66.83) [a]
8.	*Bacillus subtilis*	1.37	81.85 (64.79) [a]
9.	Control	7.53	0.00 (2.87) [g]
	C.D. (P=0.05)		2.24

* Mean of three replications
(Data in parentheses are arc sine transformed values)
In a column, means followed by common letter(s) are not significantly different at 5 % level by DMRT

Table 2 : Efficacy of rice phylloplane microflora against sporulation of *S. oryzae*

Sr. No.	Phylloplane microflora	Sporulation ($\times10^5$/ml)*	Per cent sporulation inhibition*
1.	*Aspergillus flavus*	3.75	67.62 (55.32) [c]
2.	*Aspergillus niger*	4.58	60.46 (51.04) [d]
3.	*Aspergillus glaucus*	5.25	54.69 (47.69) [e]
4.	*Penicillium* sp.	8.75	24.63 (29.73) [g]
5.	*Curvularia* sp.	5.83	49.63 (44.79) [f]
6.	*Cladosporium* sp.	5.00	56.88 (48.96) [de]
7.	*Pseudomonas fluorescence*	1.92	83.46 (66.01) [a]
8.	*Bacillus subtilis*	2.42	79.14 (62.83) [b]
9.	Control	11.58	0.00 (2.87) [h]
	C.D. (P=0.05)		2.56

* Mean of three replications
(Data in parentheses are arc sine transformed values)
In a column, means followed by common letter(s) are not significantly different at 5% level by DMRT

Table 3 : Efficacy of rice phylloplane microflora against sheath rot incidence under pot culture conditions

Sr. No.	Phylloplane microflora	Disease index*	Per cent disease reduction*
1.	*Aspergillus flavus*	405.33	44.42 (41.80) [c]
2.	*Aspergillus niger*	421.33	42.23(40.52) [cd]
3.	*Aspergillus glaucus*	448.00	38.58 (38.40) [d]
4.	*Penicillium* sp.	578.67	20.66 (27.03) [f]
5.	*Curvularia* sp.	489.33	32.91 (35.00) [e]
6.	*Cladosporium* sp.	434.67	40.40 (39.46) [cd]
7.	*Pseudomonas fluorescence*	229.33	68.56 (55.90) [a]
8.	*Bacillus subtilis*	269.33	63.07 (52.58) [b]
9.	Control	729.33	0.00 (5.74) [g]
	C.D. (P=0.05)		2.39

*Mean of three replications
(Data in parentheses are arc sine transformed values)
In a column, means followed by common letter(s) are not significantly different at 5 % level by DMRT

Rice phylloplane microflora were screened *in vitro* for their antagonistic activity against *S. oryzae. P. fluorescens* exhibited the minimum (1.17cm) mycelial growth as against 7.53cm in the control which accounted for the maximum inhibition of 84.51 per cent and it was on par with *B. subtilis* (81.85%). This was followed by *A. flavus, A. niger* and *Cladosporium* sp. which caused 60.63, 54.01 and 52.65 per cent growth inhibition, respectively. The minimum (27.48%) growth inhibition was shown by *Penicillium* sp. (Table 1).

All the rice phylloplane fungi and bacteria tested, were inhibitory to the sporulation of *S. oryzae* at varying levels. *P. fluorescens* showed the minimum ($1.92x10^5$ conidia/ml) sporulation as against $11.58x10^5$ conidia/ml in the control and it registered the maximum (83.46%) inhibition followed by *B. subtilis, A. flavus, A. niger, Cladosporium* sp. and *A. glaucus* which recorded 79.14, 67.62, 60.46, 56.88 and 54.69 per cent inhibition, respectively (Table 2).

P. fluorescens showed the maximum disease reduction to an extent of 68.56 per cent and recorded the disease index of 229.33 as against 729.33 in the control. This was followed by *B. subtilis, A. flavus, A. niger* and *Cladosporium* sp. which, respectively recorded 63.07, 44.42, 42.23 and 40.40 per cent reduction in the disease intensity. *Penicillium* sp. recorded the least (20.66%) disease reduction (Table 3).

Rai *et al*. (1988) isolated *A. flavus, A. niger, Alternaria alternata, Cladosporium cladosporioides, Curvularia lunata* and *Fusarium oxysporum* from rice plant surface. Manonmani (1999) from this laboratory reported that *Curvularia* sp., *Fusarium* sp., *A. niger, A. flavus, Rhizopus* sp. and *Bacillus* sp. were isolated from ADT 36 rice phylloplane. Sarala (2000) also isolated *A. nigher, A. flavus, Penicillium* sp., *Rhizopus* sp., *Trichoderma* sp., *Bacillus subtilis* and *P. fluorescens* from ADT 36 rice l eaves. Viswanathan and Narayanasamy (1990) found that an isolate of *Bipolaris zeicola* (*Cochliobolus carbonum*) from the phylloplane of IR 20 was inhibited the growth of *S. oryzae*. The undiluted culture filtrate of *B. zeicola* was also inhibitory. Manonmani (1999) observed that the rice phylloplane fungi *Curvularia* sp. and *Fusarium* sp. were significantly the most effective in inhibiting the mycelial growth of *S. oryzae* followed by *A. flavus*. The present investigation corrobrates with the findings of Sarala (2000) who found that *B. subtilis* recorded the maximum on mycelial growth of *S. oryzae* followed by *P. fluorescens, A. flavus* and *Penicillium* sp. which were all isolated from rice phylloplane.

In the present study, *P. fluorescens* isolated from rice phylloplane was highly effective in reducing sheath rot intensity followed by *B. subtilis, A. flavus, A. niger* and C*ladosporium* sp. Narassimmaraj (1991) reported that spraying of *Bacillus* sp. suspension was effective in controlling rice sheath rot disease. Pre-inoculation spraying of *P. fluorescens* and *Bacillus* sp. were effective in reducing the sheath rot intensity as reported by Pandiaraja Kumar (1992). Radhika (1994) observed that pre-inoculation spraying of *P. fluorescens*; *B. subtilis* and *Trichoderma viride* were effective in reducing sheath rot intensity. Manonmani (1999) also observed that gypsum based formulations of *P. fluorescens* (pf-1) and *B. subtilis* had the maximum efficacy in the management of the rice sheath rot disease.

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