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Eco-friendly Approaches for the Management of Sheath Rot (*Sarocladium oryzae,* (Sawada)) in Rice

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ABSTRACT

Fungicide/antibiotic resistance is an increasing problem leading to increased incidence of certain diseases like sheath rot in rice which has assumed as a major disease in all rice growing areas of world. Present investigation revealed that out of eighteen rice genotypes, Masuri was found resistant to *Sarocladium oryzae* while NTP-98B and NTP-98A were found moderately resistant. Jaya, TN-1, GR-6, GR-4, IR-66, IR-50, IR-28, IR-20 and CR-138-928 were highly susceptible to sheath rot. Seed bacterization and seed treatment with fungal biocides inhibited the growth of the *S. oryzae*. Among bacterial biocides *Pseudomonas fluorescens* and fungal biocides *Trichoderma viride* under *in vitro* and *Bacillus subtilis, P. fluorescens* and *T. viride* under *in vivo* conditions were found most effective in controlling *S. oryzae* the causal agent of sheath rot disease of rice.

Key words: Disease incidence, Genotypes, *Pseudomonas fluorescens*, *Trichoderma viride,* Rice.

Rice (*Oryza sativa* L.) is the staple food for more than half of the world population and approximately 90 per cent of the world's rice production is from Asia (FAO, 2012). The productivity of rice is threatened by a number of diseases attacking from nursery to harvest. Pests and diseases cause about 35 - 40 per cent annual yield loss in rice (Srinivasachary *et al*., 2002). Sheath rot of rice caused by *Sarocladium oryzae* has been reported to cause up to 1.7 per cent loss in grain yield with one per cent disease intensity in uplands. The disease affects the quality of the rice grain and severe infection reduces the yield up to 85 per cent (Prabhakaran *et al.,* 1973; Reddy, 1991). Keeping in view of the importance of the disease, experiments were planned for the management of disease through identification of resistant varieties and use of fungal antagonists as an integral part of Integrated Disease Management.

MATERIAL AND METHODS

i. Screening of rice germplasm against *S.oryzae*

Pot experiment was conducted at National Agricultural Research Project net house, Gujarat Agricultural University, Navsari by transplanting eighteen germplasm lines at the rate of three tillers per pot and three replications were maintained. Two experimental sets were maintained out of which one set of pots were inoculated with S.oryzae at boot leaf stage with single grain inoculation method and another set of pots was kept uninoculated as control. Observations were recorded on randomly selected nine tillers in each line using 0-9 scale of Standard Evaluation System (SES) for rice sheath rot (IRRI, 1978) (Table.1). The resistance reaction of various lines to disease was determined based on the Per cent Disease Index (PDI) worked out using the formula PDI = (1xA) + (3xB) + (5xC) + (7xD) + (9xE), where A= per cent of tillers having grade =1; B= per cent of tillers having grade =3; C= per cent of tillers having grade =5; D= per cent of tillers having grade = 7; E= per cent of tillers having grade =9 (Narayanaswamy and Viswanath,1990) (Table. 2).

i.Effect of biocides as seed treatment

Seven biological agents consisting of five fungal and two bacterial cultures (Table. 3) were used throughout the study and the details were given in Table. 1.

The bacterial biocides were cultured in nutrient broth media (Peptone 5 g, Beef extract 3 g, NaCl 5 g, $MgSO_4$ 0.5 g, distilled water 1000 ml) and the fungal biocides were cultured in 250 ml conical flask containing 100 ml of congored medium (3 g Glucose, 1 g NH_4NO_3, 0.9 K_2HPO_4, 0.5 g KCl,

0.2 g $MgSO_4 \cdot 7H_2O$, 0.25 g Chlorompenicol (1 capsule), 0.15 ml rose bengal and 1000 ml distilled water) medium.

After 15 days of incubation at room temperature (27 ± 2°C), the spore suspension of 10 x 10^6 spore ml^{-1} fungal biocides were prepared. After 72 h of bacterial growth, the bacteria were scraped and suspended in 0.1 M $MgSO_4$ to which one per cent (W/V) Sodium Carboxy methyl cellulose (CMC) was added resulting in a slurry. To know the effect of biocides as seed treatment, seed treatments with biocides were carried as pre-inoculation of biocide to pathogen inoculation and post inoculation of biocide to pre-inoculated pathogen. Seeds treated with solution of Na-CMC and $MgSO_4$ (without bio agent) served as control (Laha *et al*., 1992).

a.Pre inoculation of biocide as seed treatment followed by pathogen inoculation: The surface sterilized rice seeds (50g) were soaked in various culture filters of biocides for 24 h followed by soaking of seeds in cultural filtrate of *S. oryzae* for 24 h.

b.Post inoculation of biocide as seed treatment to the pathogen pre-inoculated seed: Fifty grams of rice seeds were surface sterilized and soaked in culture filtrates of *S. oryzae* for 24h. These inoculated seeds were soaked in previously prepared filtrates of microbial bioagents for 24 h.

c.Seed treatment with biocides: Fifty grams of rice seeds were soaked in previously prepared filtrates of microbial bioagents for 24 h.

The efficacy of the antagonists as seeds treatment was tested using seed germination tests (ISTA, 1976). Three layers of white sterile blotter paper pre-soaked with sterile distilled water were placed in the high quality transparent plastic Petri dishes and 25 treated seeds were placed at equidistance i.e., 16 seeds at outer periphery, eight seeds in inner periphery and one seed in center. All the seeded Petri dishes were kept for germination at 27± 2 ^{0}c for five to ten days in an incubator. Four replications were maintained with each biocide. After a specified period of time (5-10 days) the seedling length was measured and mean seedling length was calculated. Germination percentage and Seed Vigour Index (SVI) were calculated using the formula given by Abul-Baki and Anderson (1973) and Hosain *et al.* (2006).

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Number of seeds on the tray}} \times 100$$

S.V.I = Germination (%) × Seedling length (Total seedling length=shoot height +Root length)

i.Antagonistic activities of biocides against *S. oryzae* under *in vitro* conditions

The antagonistic activities of the biocides were studied using dual culture technique (Dennis and Webster, 1971). The Petri dishes containing PDA without any bio agent but with culture *S.*

Table 1. The Standard Evaluation System (SES) for rice sheath rot

Scale		Incidence of severely infected tillers	Description
0	-	No incidence	No disease incidence
1	-	< 1 %	Small, brown lesions on boot leaf sheath and panicle emergence normal
3	-	1 - 5 %	Lesions enlarge or coalesce and covered about 5 per cent of leaf sheath and panicle emergence normal
5	-	6 - 25 %	Lesions covered about 6-15 per cent of leaf sheath area and 75 per cent of panicle emergence exerted
7	-	26 - 50 %	Lesions covered about 16-50 per cent of leaf sheath area and 50 per cent of panicle exerted
9	-	51 - 100 %	Lesions covered more than 50 per cent of leaf sheath and panicle emergence completely affected or only 25 per cent of panicle exerted

Table 2. Germplasam reaction for rice sheath rot determined based on per ce t Disease index.

S. No.	Grade	Symbol	Reaction
1	0-99	H	Highly Resistant
2	100-199	R	Resistant
3	200-299	MR	Moderately Resistant
4	300-499	MS	Moderately Susceptible
5	500-699	S	Susceptible
6	700-899	HS	Highly Susceptible

Table 3. Biocides used for control of sheath rot of rice *in vitro.*

S. No.	Name of biocide	Source of culture
	Fungal bioagents	
1.	*Trichoderma viride* (TV)	I.A.R.I., New Delhi
2.	*T. harzianum* (TH)	S.B.I., Coimbatore
3.	*T. longibrachyatum* (TL)	I.A.R.I., New Delhi
4	*Chaetomium globosum* (CG)	I.A.R.I., New Delhi
5.	*Aspergillus niger* (AN)	G.A.U., Navsari
	Bacterial bioagents	
6.	*Pseudomonas fluorescens* (Psf)	G.A.U., Navsari
7.	*Bacillus subtilis* (BS)	G.A.U., Navsari

oryzae alone served as control (Laha *et al*., 1992). The inoculation of pathogen was followed as per the standard procedures and the Petri dishes were incubated at 27± 2^0C with 95±5 per cent relative humidity. The biological agent's fungi were grown on congored agar medium for 5-7 days and biological agent's bacteria were grown on nutrient agar medium for 72 h. The *S .oryzae* was cultured on wheat flour agar medium for 20 days. The 5 mm culture block of pathogen as well as fungal and bacteriological bioagents were cut aseptically from the outer periphery and placed opposite to each other, 60 mm apart in Petri dish. In the control condition only pathogen was placed in the Petri dish. The Petri dishes were allowed for incubated at room temperature (27 ± 2°C). The percent growth inhibition was calculated according to the formula of Asalmol *et al*. (1990).

$$\text{Per cent growth inhibition (I)} = \frac{C - T}{C} \times 100$$

Where,

I = Per cent inhibition
C = Growth in control after incubation
T = Growth in treatment after incubation

ii. Antagonistic activities of bioagents against *S.oryzae* under *in vivo* conditions

Field trial was laid with rice cultivar Jaya at Paddy Research Station, National Agricultural Research Project, Navsari. The trial was conducted in RBD with eight treatments in a plot of 5.4 x 3.6 m, with 15 x 10 cm spacing with recommended fertilizer dosages was replicated thrice. The pathogen was multiplied on paddy chaffy grains in 250 ml conical flasks when 25 to 50 ml of water was added to 200 g of chaffy grains for sterilization. The flasks were inoculated with mycelia discs (15 mm) of *S. oryzae*. The chaffy grains fully covered with fungus were used for inoculating rice plants after 20 days. The first spray with the bio-control agent was given at 50 per cent flowering. Pathogen inoculation was done after 7-10 days of bio-control agent's spray. Chaffy grains with well developed mycelial growth were placed in between boot leaf sheath and panicle in each tiller and covered with moist cotton. The spore suspension of fungal bioagents with 10 x 10^6 CFU ml^{-1} and the bacterial bioagent with 6 x 10^7 CFU ml^{-1} was used. The data pertaining to PDI, Yield, health and discoloured

Table 4 . Screening of germplasm against sheath rot of rice .

S.No.	Germplasm lines	PDI (Per cent disease index)	Reaction
1.	TN-1	39 (86.6)	HS
2.	Jaya	36 (80.0)	HS
3.	GR-4	36 (80.0)	HS
4.	IR-50	35 (77.7)	HS
5.	IR-28	35 (77.7)	HS
6.	IR-22	34 (75.5)	HS
7.	GR-6	34 (75.5)	HS
8.	CR-138-928	34 (75.5)	HS
9.	IR-66	33 (73.3)	HS
10.	GR-3	31 (68.8)	S
11.	GR-11	31 (68.8)	S
12.	Gurjari	30 (66.6)	S
13.	Narmada	24 (53.3)	S
14.	Nawagam	15 (33.3)	MS
15.	GR-3 x IET-9-9865	15 (33.3)	MS
16.	NTP-98B	11 (24.4)	MR
17.	NTP-98A	10 (22.2)	MR
18.	Masuri	00 (00.0)	R
19.	Control	00 (00.0)	-
	S. Em. ±	0.39	
	C.D. at 5 %	10.18	
	C.V. %	8.12	

* Figures in parenthesis are Arcsine transformed values
R= Resistant; MR= Moderately Resistant;
MS= Moderately Susceptible; HS= Highly Susceptible; S= Susceptible

grains and other parameters were recorded and discussed.

RESULTS AND DISCUSSION

i.Screening of rice germplasm against *S.oryzae*

Among the lines tested none of the lines were found immune. Masuri was the only line with resistant reaction having 0 grade. Among the other lines, NTB-98B (11), NTP-98A (10) had mderately resistant reaction, Nawagam (15), GR-3 X IET-9-9865 (15) lines were moderately susceptible, GR-3 (31), GR-11 (31), Gurjari (30), Narmada (24) were susceptible and nine cultures (TN-1, Jaya, GR-4, IR-50, IR-28, IR-22, GR-6, CR-138-928 and IR-66 were highly susceptible (Table 4). Singh and Dodan (1995) reported that Kalanamak, Masuri and kasha are resistant to sheath rot while Jaya and co-43 were susceptible. Manibhusanrao (1996) reported that Pankaj and Masuri as resistant while IR- 50 and Kannage as susceptible to sheath rot. Biswas and Samajpathi (1999) reported that IET-2815 (Sashvasree), IR-42 and NC- 492 (Sabita) as resistant to Sheath rot, while IR-20, IR-36 and IR-50 as susceptible.

i.Effect of seed treatment of biocides on *S. oryzae*

a. Pre-inoculation of biocide as seed treatment followed by pathogen inoculation: Significant differences were observed between treatments pertaining to germination percentage and also mean shoot length, however, differences between treatments were not significant pertaining to the root length. Among all the treatments, highest germination percentage (99.0) was recorded with pre-inoculation seed treatment of *C. globosum* and *T. longibrachiatum* followed by *B. subtilis* and *A. Niger* (98.0). Germination was highly

Table 5. Effect of biocides seed treatment on rice (cultivar Jaya) seed against *S. oryzae.*

S No	Biocide	Biocide treatment				Pre inoculation of biocide to pathogen				Post inoculation of biocide to pathogen			
		Mean Germination (%)	Mean shoot length (cm)	Mean root length (cm)	Vigour Index	Mean Germination (%)	Mean shoot length (cm)	Mean root length (cm)	Vigour Index	Mean Germination (%)	Mean shoot length (cm)	Mean root length (cm)	Vigour Index
1	*Bacillus subtilis*	99.0	1.3	1.0	228.0	98.0	1.0	1.0	207.0	99.0	1.5	0.7	218.0
2	*Pseudomonas Fluorescens*	98.0	1.5	0.7	216.0	94.0	1.5	0.7	206.0	96.0	1.3	0.6	185.0
3	*Chaetomium globosum*	95.0	1.3	0.4	162.0	99.0	1.4	0.5	188.0	99.0	1.0	0.3	129.0
4	*Trichoderma l ongibrachiatum*	90.0	1.2	0.7	171.0	99.0	0.5	0.3	79.0	98.0	1.2	0.6	173.0
5	*Aspergillus niger*	90.0	0.7	0.3	90.0	98.0	1.4	0.4	176.0	97.0	1.3	0.7	197.0
6	*T, viride*	89.0	0.8	0.5	116.0	97.0	1.3	0.7	194.0	96.0	0.8	0.4	118.0
7	*T. harzianum*	80.0	1.0	0.4	112.0	70.0	1.0	0.4	98.0	60.0	0.7	0.3	58.0
8	Control (untreated)	51.0	0.7	0.4	56.0	52.0	1.0	0.4	73.0	50.0	0.7	0.4	54.0
	S.Em±	0.9	0.2	0.0	1.38	0.8	0.0	0.0	1.66	0.81	0.15	0.06	1.38
	CD at 5%	2.5	NS	NS	6.14	0.2	0.1	NS	4.99	2.37	NS	NS	6.14
	CV%	1.7	22.4	37.1	6.23	1.5	5.3	37.1	6.37	1.62	24.7	23.0	6.23

affected with pre-inoculation seed treatment with *T. harzianum* with a germination percentage of 70. The mean maximum shoot length was recorded in *P. fluorescens* (1.50 cm) and maximum mean root length was recorded in *B. subtilis* treatment (1.0 cm) (Table. 5). Among the different treatments, even though *T. longibrachiatum* recorded highest mean germination percentage (99.0) but mean root and shoot length are severely affected and hence recorded lowest vigour index (79.0). Among the different treatments highest seed vigour index was recorded with *B. subtilis* (207.0) followed by *P. flurescens* (206.0).

b. Post inoculation of biocide as seed treatment to the pathogen pre-inoculated seed :

Significant differences were observed pertaining to germination percentage among treatments and not significant related to root and shoot length. Among the treatments tested, highest germination percentage (99.0) was recorded with post-inoculation seed treatment of *T. harzianum* and *B. subtilis* followed by (98%) with *T. longibrachiatum.* This was followed by *T. viride*, *A. niger* and *C. globosum* 98.0, 97.0 and 96.0 percentages respectively. The highest mean shoot (1.5 cm) and root lengths (0.70 cm) were observed in case of *B. subtilis* followed by *P. fluorescens* and *A. Niger.* Highest Seed Vigour Index (218.0) was also recorded with *B. subtilis* followed by *A. niger* (197.0) and *P. fluorescens* (185.0) and the least was recorded with *T. harzianum* (58.0).

c. Seed treatment with biocides: Among the two bacterial biocides, *B. subtilis* and *P. fluorescens* gave significantly highest germination percentage 99.0 and 98.0

Table 6. Biological control of sheath rot pathogen (*S. oryaze*) *in vitro*.

S. No.	Treatment	Bio control agent's	Diameter of fungus growth (mm)	Mean Growth Inhibition Zone (%)
1.	T1	*Bacillus subtilis*	36.0	40.0
2.	T2	*Pseudomonas fluorescens*	42.3	47.0
3.	T3	*Trichoderma viride*	76.5	85.0
4.	T4	*T. longibrachiatum*	69.3	77.0
5.	T5	*T. harzianum*	56.7	63.0
6.	T6	*Chaetomium globosum*	22.5	25.0
7.	T7	*Aspergillus niger*	49.5	55.0
8.	T8	*A. flavus*	56.3	60.0
9.	T9	*Gliocladium virens*	18.0	20.0
10.	T10	Control	90.0	0.0
S. Em. ±				1.12
C.D. at 5 %				3.70
C.V. %				5.31

Table 7. Effect of biocides as foliar spray on sheath rot pathogen *S. oryaze in vivo*.

Treatment/ Biocide	*Rabi* 2000					
	PDI %	1000 grain wt (g)	Grain yield (q/ha)	Mean Per cent of grains		
				Healthy	Dis-coloured	Unfilled
T1- *Bacillus subtilis*	3.00	31.00	32.50	87.33	9.01	3.66
T2- *Pseudomonas Fluorescens*	0.66	33.33	40.00	83.66	9.34	7.00
T3- *Trichoderma viride*	1.70	35.00	39.50	82.33	10.07	7.60
T4- *T. longibrachyatum*	7.00	31.67	26.50	81.33	10.34	8.33
T5- *T. Harzianum*	7.48	29.67	33.00	77.83	13.17	9.00
T6- *Chaetomium Globosum*	21.00	29.00	23.00	76.00	14.00	10.00
T7- *A. niger*	29.00	29.33	30.00	66.33	22.67	11.00
T8- *Aspergillus flavus*	30.00	28.00	25.00	54.60	33.40	12.00
T9- *Gliocladium virens*	46.66	29.00	19.00	48.66	37.34	14.00
T10- Control	61.33	30.00	18.00	38.66	45.34	16.00
S. Em. ±	3.00	1.82	0.47	1.82	0.49	0.55
C.D. at 5 %	8.86	5.38	1.46	5.38	1.46	1.63
C.V. %	24.12	10.35	10.42	10.35	10.42	10.75

than the fungal biocides and control. Among the seven fungal biocides, *C. globosum* gave significantly highest germination percentage (95.0) as compared rest of fungal biocides as well as control. *T. longibrachiatum, A. niger and T. viride* recorded 90.0, 90.0 and 89.0 percentages respectively. They were at par with each other but gave significantly higher germination percentage than control.

Pseudomonas fluorescens gave the higher mean shoot length (1.50 cm) whereas *B. subtilis* gave the higher mean root length (1.0 cm) as compared to control. Among the fungal biocides, the highest mean shoot and root length were

recorded in *C. globosum* (1.3 cm and 0.4 cm) and *T. longibrachiatum* (1.2 cm and 0.70 cm) treated seeds, which were superior over all the other fungal biocides and was followed by *C. globosum* and *T. harzianum*.

The antagonistic interaction of some soil fungi of *T. viride*, *T. longibrachiatum*, *T. harzianum*, *A. flavus*, *A. niger*, *G. virens*, *C. globosum*, *P. fluorescens a*nd *B. subtilis* against the sheath rot pathogen was studied by Panneerselvam and Saravamuthu (1996) *in vitro* in dual culture and in Petri dishes on nutrient medium amended with culture filtrates of the test fungi. The percentage growth inhibition of *S. oryzae* by the antagonistic fungi were reported maximum with *T. viride* (Srinivas and Ramakrishna, 2003), *Gliocladium* sp., (Sakthivel and Gnanamanickam, 1986), *P. fluorescens (*Manibhushanrao, 1996). Present findings are also confirming the previous reports and shows that *A. niger*, *T. viride*, *C. globosum*, *P. fluorescens* and *B. subtilis* were effective in increasing germination, mean root and shoot lengths when used as biocidal seed treatment (Table 5)

iii. Antagonistic activities of biocides against *S.oryzae* under *in vitro* conditions

Among five fungal and two bacterial biocides tested, fungal biocides *T. viride* and T. *longibrachiatum* have 85.0, 77.0 per cent growth inhibition and were found significantly superior antagonists to all other bio agents. *T. harzianum* had growth inhibition of 63.0 per cent followed by *A. flavus* (60.0 %), *C. globosum* (25.0 %) and *G. virens* (20.0 %) were slightly antagonistic to *S. oryzae*. Among the bacterial biocides, the *P. fluorescens* (47 per cent) was better than the *B. subtilis* (40.0 %) was found superior to *B. subtilis* (*40.0* %) however, these two bacterial biocides were significantly superior in inhibiting the growth of *S. oryzae* than the *C. globosum* and *G. virens*.

The antagonistic interaction of some soil fungi of *T. viride*, *T. longibrachiatum*, *T. harzianum*, *A. flavus*, *A. niger*, *G. virens*, *C. globosum*, *P. fluorescens a*nd *B. subtilis* against the sheath rot pathogen was studied by Panneerselvam and Saravamuthu (1996) *in vitro* in dual culture and in Petri dishes on nutrient medium amended with culture filtrates of the test fungi. Inhibition of *S. oryzae* growth by the antagonistic fungi were observed maximum with *T. viride* followed by *Gliocladium* sp. (Sakthivel and Gnanamanickam,1986). Similarly *P. fluorescens* checked the growth of *S. oryaze* in *in vitro* and *in vivo* (Sundarmurthy *et al*. 2013) and the present findings are in accordance with the earlier reports (Table.6).

iv. Antagonistic activities of biocides against *S. oryzae* under *in vivo* conditions

The results indicated that all the biocides significantly reduced the per cent disease intensity as compared to control. Among all the treatments *P. fluorescens*, *T. viride, B. subtilis, T. longibrachyatum* and *C. globosum* significantly reduced PDI as compared to the rest of biocides and control (Table 7).

The 1000 g grain weight was found highest in *T. viride* treated plots. only *P.fluorescens* treatment had shown significantly higher grain yield (40.00 q ha^{-1}) followed by *T. viride* (39.50 q ha^{-1}), *T. harzianum* (33.00 q ha^{-1}), *Bacillus subtilis* (32.50 q ha^{-1}) treatment and were at par with each other.

Highest number of healthy grains were recorded in *B. subtilis* treated plots (87.33 %) and was followed by *P. fluorescens* (83.66 %), *T.viride* (82.33 %) and T. *longibrachiatum* (81.33 %) sprayed plots and were at par with each other compared to others. The minimum discoloured grains were obtained in *B. subtilis* sprayed plots (9.01 %) followed by *P. fluorescens* (9.34 %), *T. viride* (10.07 %) and *T. harzianum* (13.17 %) sprayed plots and were at par with each other compared to rest of the treatments. Less unfilled grains were found in case of *B. subtilis* (3.66 %) treated plots than the rest of treatments.

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