Evaluation of foliar sprays of biocontrol agents, botanicals and fungicides for the production of healthy rice seed in the coastal ecosystem

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Abstract

The effect of foliar spraying with *Pseudomonas fluorescens* + butter milk (0.5 %), *P. fluorescens* (0.5 %), neem seed kernel extract (5 %) neem oil (3 %), Copper hydroxide (0.25 %), Propiconazole (0.1 %), Carbendazim (0.1 %) + Mancozeb (0.1 %) at booting and again at 50 % flowering against major diseases of rice during *Rabi* season was investigated in comparison with water spray under natural condition. Among them, *P. fluorescens* + butter milk recorded minimum sheath rot, false smut and grain discolouration at par with *P. fluorescens*, Propiconazole and Carbendazim + Mancozeb and significantly higher seed yield. Six fungal species including *Aspergillus niger*, *A. flavus*, *Bipolaris oryzae*, *Curvularia lunata*, *Fusarium moniliforme* and *Sarocladium oryzae* were isolated from the seed samples after harvest and six months of storage in gunny bags under ambient condition. The population of storage fungi increased slightly on contrary to pathogenic fungi with storage. Seeds contaminated with relatively low level of mycoflora invariability exhibited more than 80 per cent germination after six months of storage. The seed samples collected from either *P. fluorescens* + butter milk (0.5 %) or *P. fluorescens* (0.5 %) sprayed plants recorded minimum mycoflora and maximum germination after six months of storage.

Key words: Butter milk, fungicide, neem products, *Pseudomonas fluorescens*, seed mycoflora

1. Introduction

Rice (*Oryza sativa* L.) is the staple food for more than two-thirds of the Indian population. India is the second largest producer of rice with an annual production of 117.5 million tonnes annually contributing approximately 40% to the total food grain production (FAOSTAT, 2018). As seed is the critical determinant of sustainable increase in agricultural production, production and supply of quality seeds in sufficient quantities at affordable prices to the farmer is needed for raising productivity. Pathak *et al.* (2020) reported that majority of the farmers are using seeds saved under their own custody and non-availability of good quality seeds continues to be a problem for the farmers

for cultivation despite a fair institutional framework for seed production both in the public and private sector. Mew and Gonzales (2002) reported that more than 90% of the rice seed stocks for planting in tropical Asia are the farmers' own seed saved from previous rice harvest. The *Alternaria alternata*, *A. padwickii*, *Bipolaris oryzae*, *Curvularia lunata*, *Epicoccum nigrum*, *Fusarium moniliforme*, *F. semitectum*, *F. solani*, *Microdochium oryzae*, *Nigrospora oryzae*, *Pyricularia oryzae*, *Rhizoctonia oryzae* and *Sarocladium oryzae* found to be associated with rice seed externally as well as internally affect seed germination, seedling establishment and grain quality (Reddy *et al.*, 2004; Akila

and Ebenezer, 2009). Apart from this, *B. oryzae*, *C. lunata*, *F. moniliforme*, *M. oryzae*, *P. oryzae* and *S. oryzae* incites brown spot, black kernel, bakanae, leaf scald, blast, and sheath rot respectively in the standing crop (Ou, 1985). Thus, seed health is a well-recognized factor for establishment of normal seedlings of desired plant population for good harvest. In general, rice growing ecosystem of the coastal areas and prevailing weather factors during the *Rabi* season (October to January) predispose epidemic outbreak of blast, sheath rot, false smut and grain discolouration diseases every year and pose heavy economic loss to the farmers. To surmount these problems and to ensure food security, disease management strategies evolved from various phytopathological studies have been framed as packages of practices and recommended to the farmers. However, cloudy and humid weather during the heading stage of *rabi* crop causes severe grain discolouration due to the contamination of seeds with mycoflora present on different parts of plants during pre- and post-harvest stage, which in turn reduces market value of seed and fetches low price and sometime is rejected by procurement agencies. This warrants the data on the incidence and diversity of seed microflora for predicting the extent of post-harvest infection and subsequent deterioration of rice seed. Taking clue from this, the foliar sprays of biocontrol agents, botanicals and fungicides recommended for managing rice diseases were evaluated for the production of healthy seeds in the coastal ecosystem.

2. Materials and Methods

The field trials were conducted under natural condition at Pandit Jawaharlal Nehru College of Agriculture and Research Institute, Karaikal, U.T of Puducherry during *rabi* season (October to January) for two years with eight treatments and three replications in a randomized block design. The highly susceptible ruling cultivar, BPT 5204 was raised in plots of 5 m × 2 m with a spacing of 20 × 10 cm by adopting standard agronomic practices. The foliar sprays with *P. fluorescens* TNAU talc (0.5 %), *P. fluorescens* TNAU talc + butter milk (0.5 %), neem seed kernel extract (5%) neem oil (3%), Copper hydroxide (0.25 %), Propiconazole (0.1 %), Carbendazim (0.1 %) + Mancozeb (0.1%) were given once at booting and again at 50 % flowering stage. The water sprayed plants served as control. The natural incidence of sheath rot, false smut and grain discolouration was recorded as per IRRI (2002). The

hills from the central one square metre area were marked and number of healthy and diseased tillers/panicles in each hill was counted for calculating the per cent incidence of sheath rot and false smut. The hills in the central one square meter area of each plot were harvested separately and apparently healthy and spotted seeds were separated and presented as percentage of grain discolouration. The moisture of the seed was brought down to 12 per cent and seed yield was recorded.

The seeds harvested from each plot were subjected to roll towel method and standard blotter paper as per ISTA (2015) six months of storage in gunny bags under room temperature to assess seed viability. The observation on seed germination (%) was recorded in the blotter paper 14 days after incubation. The root length and shoot length were also measured and vigour index was worked out as Vigour index = Germination (%) × Total seedling length (cm) (Abdul – Baki and Anderson, 1973). The mycoflora associated with the seeds were isolated by agar plate method after harvest and six months of storage (Agarwal and Sinclair, 1993). The observations on fungal growth on the seed in the agar plate were recorded at two days interval of incubation for 4-5 days and frequency occurrence of mycoflora was calculated. The identity of different mycoflora was confirmed by comparing cultural and morphological characters as per respective description cited by Mew and Misra (1994).

3. Results and Discussion

The data presented in Table 1 revealed that plants received the foliar sprays of biocontrol agents, botanicals and fungicides showed variable effects on reduction of sheath rot, false smut and grain discolouration under field condition. Of which, *P. fluorescens* + butter milk (0.5 %) was found to be the most effective with significantly different lower incidence of sheath rot (3.11 %; 3.50 %), and recorded false smut (0.53 %; 2.00%) incidence at par with *P. fluorescens* (0.5 %), copper hydroxide (0.25 %), Propiconazole (0.1 %); and grain discolouration (6.25%; 6.67%) incidence at par with carbendazim (0.1%) + Mancozeb (0.1%). The plants received water spray recorded higher incidence of sheath rot (18.88 %; 18.14%), false smut (3.50 %; 4.00%) and grain discolouration (32.71 %; 34.77%). Regarding seed yield, *P. fluorescens* + butter milk (0.5 %) performed well with maximum seed yield (6.42 t ha⁻¹; 6.15 t ha⁻¹) as against control with seed yield of

3.40 t ha⁻¹ and 3.20 t ha⁻¹, respectively during trial I and II. Foliar spraying of *P. fluorescens* (0.5 %) was found to be the next best treatment observed to reduce sheath rot (7.25 %; 4.00%), false smut (0.67 %; 2.22%) and grain discolouration (15.00%; 11.50%) incidences and seed yield (5.40 t ha⁻¹; 5.34 t ha⁻¹) (Table 1). Our results are in agreement with the studies conducted by Thiribhuvanamala *et al.* (2013) and Vinodkumar *et al.* (2018) who reported that foliar spraying of plant growth promoting rhizobacteria along with butter milk not only reduced the tobacco streak virus incidence in cotton but also promoted plant growth and yield. Further, Vinodkumar *et al.* (2018) stated that when buttermilk was used as a carrier base for application, the biocontrol agent effectively colonizes phylloplane of the plant and produce anti-microbial peptides and fatty acids to curb pathogens. The efficacy of talc-based formulation of *P. fluorescens* developed in TNAU was also proved against major rice diseases by Akila and Ebinezar (2009) and Jeyalakshmi *et al.* (2010).

In the present study, minimum incidence of grain discolouration (5.75%; 4.00%) was observed in Carbendazim + Mancozeb treated plants. Similar to this, Bag *et al.* (2010) documented good performance of carbendazim + mancozeb in reducing grain discolouration and protecting the grain yield of rice. The efficacy of propiconazole (0.1%) or copper hydroxide (0.25%) spray was found to be at par with *P. fluorescens* + butter milk (0.5%) and *P. fluorescens* (0.5%) in reducing false smut in trial I and II. The plants received either Propiconazole (0.1%) or Copper hydroxide (0.25%) spray was totally free from false smut in trial I and recorded minimum incidence in trial II. Bagga and Kaur (2006) found that propiconazole application during booting and heading stage effectively reduced the number of false smut balls in the harvested rice. Identical result was documented by Karthikeyan and Jeyaraj (2010), where in foliar spraying of copper hydroxide effectively controlled false smut disease. Lore *et al.* (2007) suggested Propiconazole as the most effective fungicide against sheath rot and grain discolouration diseases. Venkateswarlu and Chauhan (2005) documented that Mancozeb + Carbendazim and propiconazole were equally effective in reducing sheath rot intensity and recording maximum healthy grain yield. The results of the present study revealed that Carbendazim (0.1%) + Mancozeb (0.1%) spray was more effective against grain discolouration (5.75 %; 4.00%) and less effective against

sheath rot (8.00%; 5.55%) and false smut (1.50 %; 3.11%) in both trials and found in line with those of Bag *et al.* (2010).

Six fungal species including four field fungi viz., *Bipolaris oryzae*, *Curvularia lunata*, *Fusarium moniliforme*, and *Sarocladium oryzae* and two storage fungi viz. *Aspergillus niger*, and *A. flavus* were isolated from the seed samples after harvest and six months of storage in the gunny bags under ambient condition (Table 2). The association of mycoflora in the fresh and stored rice seeds have been reported earlier by Archana and Prakash (2013). Among the six mycoflora isolated, *B. oryzae*, *C. lunata*, *F. moniliforme* and *S. oryzae* were respectively reported to cause black kernel, foot rot, brown spot, leaf scald and sheath rot in rice (Ou, 1985). The prevalence of the isolated fungi also varied depending upon the treatment and period of storage. The mean frequency of occurrence of mycoflora ranged from 1 - 32 per cent and 0- 35 per cent, respectively in the seeds harvested from trial I and II and mycoflora load either unchanged or slightly decreased in the seeds collected from treated plants than seeds of water sprayed plants where mycoflora load increased upon storage. The *B. oryzae* was spotted only in the seed of control plants, whereas *C. lunata*, *F. moniliforme* and *S. oryzae* were absent in the seeds collected from plants sprayed with *P. fluorescens*+ butter milk (0.5%) and *P. fluorescens* (0.5%) even after six months of storage. The prevalence of *A. niger* was noticed in the seed samples of all treatments throughout the study. The results are in conformity with Purushotham *et al.* (1996). The aflatoxin producing fungus, *A. flavus* was completely absent in *P. fluorescens* + butter milk (0.5%) and *P. fluorescens* (0.5%) and Carbendazim (0.1%) + Mancozeb (0.1%) treatments throughout the study; 6 – 11 per cent was noticed in neem oil after harvest and six months of storage ; meagre presence (2 – 4 %) was observed in Propiconazole and Copper hydroxide treatments six months of storage and relatively high level (20 – 48 %) was spotted in control treatments after harvest and six months of storage.

The prevalence of field fungi decreased as the storage period increases and was generally replaced by storage fungi (Table 2). The most frequently isolated seed mycoflora in the trial I were *A. niger*, followed by *C. lunata*, *S. oryzae*, *F. moniliforme*, *A. flavus* and *B. oryzae* irrespective of the treatments tested after harvest and six months of storage. However, *S. oryzae* and *F. moniliforme* were found

Table 1. Effect of foliar sprays of biocontrol agents, botanicals and fungicides on the incidence of major diseases and seed yield of rice

Treatments	Trial I					Trial II				
	Sheath rot (PI) *	False Smut (PI) % *	Grain Discolouration (PI) % *	Seed Yield* (t ha ⁻¹)	Sheath rot (PI) % *	False Smut (PI) % *	Grain Discolouration (PI) % *	Seed Yield* (t ha ⁻¹)		
<i>Pseudomonas fluorescens</i> (0.5 %)	7.25 ^b (15.68)	0.67 ^a (4.76)	15.00 ^b (22.60)	5.40 ^b	4.00 ^a (11.54)	2.22 ^a (8.53)	11.50 ^b (19.82)	5.34 ^b		
<i>Pseudomonas fluorescens</i> + butter milk (0.5 %)	3.11 ^a (10.06)	0.53 ^a (4.13)	6.25 ^a (14.47)	6.42 ^a	3.50 ^a (10.75)	2.00 ^a (8.14)	6.67 ^a (14.89)	6.15 ^a		
Neem Seed Kernel Extract (5 %)	8.32 ^b (16.60)	1.25 ^b (6.34)	16.50 ^b (23.96)	4.30 ^d	5.92 ^b (14.93)	3.44 ^b (10.55)	18.88 ^c (25.77)	4.28 ^d		
Neem oil (3 %)	8.52 ^b (16.93)	1.75 ^b (7.53)	21.00 ^c (27.27)	4.90 ^{cd}	6.66 ^c (8.85)	5.39 (13.52)	22.96 ^d (28.63)	4.54 ^{cd}		
Copper hydroxide (0.25 %)	12.40 ^c (20.47)	0.00 ^a (2.87)	20.40 ^c (26.85)	4.60 ^d	11.84 ^e (20.12)	1.00 ^a (5.75)	24.12 ^d (29.41)	4.40 ^d		
Propiconazole (0.1 %)	9.00 ^b (17.20)	0.00 ^a (2.87)	14.50 ^b (22.38)	5.30 ^{bc}	7.67 ^d (16.07)	1.25 ^a (6.34)	14.44 ^c (22.30)	5.26 ^{bc}		
Carbendazim (0.1 %) + mancozeb (0.1 %)	8.00 ^b (16.43)	1.50 ^b (6.93)	5.75 ^a (13.86)	5.32 ^{bc}	5.55 ^b (13.58)	3.11 ^b (10.06)	4.00 ^a (11.54)	5.18 ^{bc}		
Control (water spray)	18.88 ^d (25.77)	3.50 ^c (10.76)	32.71 ^d (34.88)	3.40 ^e	18.14 ^f (25.21)	4.00 ^c (11.54)	34.77 ^e (36.42)	3.20 ^e		

*Mean of three replications. Data in parenthesis are angular transformed values. In a column, mean followed by a common letter are not significantly different at 5 % level by DMRT.

Table 2. Effect of foliar sprays of biocontrol agents, botanicals and fungicides on prevalence of mycoflora in the rice seed

Frequency of occurrence of seed mycoflora (%)													
Trial I							Trial II						
Mean (%)							Mean (%)						
AN	AF	BO	CL	FM	SO		AN	AF	BO	CL	FM	SO	
I	II	I	I	I	II	I	I	II	I	II	I	II	I
4	6	0	0	0	0	1	2	3	0	0	0	0	0
4	6	0	0	0	0	1	2	3	0	0	0	0	0
22	30	0	4	0	15	9	30	32	0	2	0	6	8
20	28	6	10	0	20	14	30	49	8	11	0	15	14
30	32	0	2	0	16	10	36	40	0	2	0	4	5
38	42	0	2	0	10	11	44	51	0	2	0	4	6
18	20	0	0	0	12	6	24	30	0	0	0	4	6
30	60	20	45	5	10	30	46	60	30	48	25	48	33
21	28	3	8	1	14	10	27	34	5	8	8	10	9

T1 - *Pseudomonas fluorescens* (0.5 %); T2 - *Pseudomonas fluorescens* + butter milk (0.5 %); T3 - Neem Seed Kernel Extract (5 %); T4 - Neem oil (3 %); T5 - Copper hydroxide (0.25 %); T6 - Propiconazole (0.1 %); T7 - Carbendazim (0.1 %) + Mancozeb (0.1 %); T8 - Control (water spray)

AN - *Aspergillus niger*; AF - *Aspergillus flavus*; BO - *Bipolaris oryzae*; CL - *Curculionella lunata*; FM - *Fusarium moniliforme*; SO - *Sarocladium oryzae*; I - After harvest and II - After 6 months of storage

to be distributed more next to *A. niger* in trial II. The results are in contradictory to Utobo *et al.* (2011) who reported a least distribution of storage fungi and the highest percentage of pathogenic fungi in discoloured rice seed after harvest. The increase in incidence of field fungi (*C. lunata*, *S. oryzae*, *F. moniliforme* and *B. oryzae*) noticed during storage in control than treatment was found in line with Surekha *et al.* (2011). The foliar spraying with fungicides and biocontrol agents reduced *A. flavus* contamination in seeds after harvest and six months of storage. Though *A. flavus* was spotted with high frequency in the untreated control (20 – 30 % and 45 – 48 %, respectively), its average frequency was low (3– 8 % and 5 – 8 %, respectively) in the fresh and stored seeds. The results clearly indicated that untreated seeds supported maximum *A. flavus* growth, if such seeds are used as grain for consumption, then the aflatoxin secreted by the fungus in the contaminated grains may pose aflatoxicosis to human beings and animals. The variation in the prevalence of seed mycoflora after harvest and storage might be due to pre- and post-maturation environmental factors and alterations in the physiology of the plant especially from flowering to seed development by the treatments.

Of the eight treatments, seeds samples collected from *P. fluorescens* + butter milk (0.5%) and *P. fluorescens* (0.5%) sprayed plants were totally free from both storage and pathogenic mycoflora in Trail II and contaminated only with *A. niger* with meagre frequency after harvest and six months of storage. Similar to this, Sharma *et al.* (2008) validated and found seed samples drawn from indigenous traditional knowledge-based treatments were comparatively free from fungi. The seed samples of Carbendazim (0.1%) + Mancozeb (0.1%), Copper hydroxide (0.25%), neem seed kernel extract (5%), Propiconazole (0.1%) and neem oil (3%) sprayed plants were shown to rank next in the order of harbouring seed mycoflora.

Regarding seed viability, seeds collected from treated plants contaminated with relatively low level of seed mycoflora exhibited more than 80 % germination and seedling vigour after six months of storage than control (Table 3). Similar observations were made by Ora *et al.* (2011), who investigated seeds with lowest mycoflora, had the highest germination per cent. The seed samples of *P. fluorescens* + butter milk (0.5%), *P. fluorescens* (0.5%),

Table 3. Effect of foliar sprays of biocontrol agents, botanicals and fungicides on seed viability and seedling vigour of rice seed

Treatments	Trial I				Trial II			
	Seed germination* (%)	Shoot length* (cm)	Root length* (cm)	Seedling vigour* (cm)	Seed germination* (%)	Shoot length* (cm)	Root length* (cm)	Seedling vigour* (cm)
<i>Pseudomonas fluorescens</i> (0.5 %)	98.00 ^a (84.23)	22.88 ^a	8.00 ^a	3026.24 ^a	98.00 ^a (84.23)	23.67 ^a	8.16 ^a	3119.34 ^a
<i>Pseudomonas fluorescens</i> + butter milk (0.5 %)	98.00 ^a (84.23)	23.58 ^a	8.20 ^a	3114.44 ^a	98.00 ^a (84.23)	24.09 ^a	8.31 ^a	3175.20 ^a
Neem Seed Kernel Extract (5 %)	88.00 ^b (70.36)	18.76 ^c	7.02 ^c	2268.64 ^d	90.00 ^b (74.99)	19.29 ^e	7.16 ^c	2380.50 ^d
Neem oil (3 %)	83.00 ^b (65.73)	19.98 ^c	6.28 ^d	2179.58 ^c	86.00 ^b (68.85)	20.51 ^c	6.32 ^d	2307.38 ^e
Copper hydroxide (0.25 %)	88.00 ^b (70.36)	19.54 ^c	7.20 ^c	2353.12 ^c	90.00 ^b (74.99)	20.05 ^d	7.30 ^c	2461.50 ^d
Propiconazole (0.1 %)	98.00 ^a (84.23)	20.48 ^c	7.16 ^b	2708.72 ^b	98.00 ^a (84.23)	21.82 ^c	7.24 ^c	2847.88 ^c
Carbendazim (0.1 %) + mancozeb (0.1 %)	98.00 ^a (84.23)	21.32 ^b	7.68 ^b	2842.00 ^b	98.00 ^a (84.23)	22.69 ^b	7.75 ^b	2983.12 ^b
Control (water spray)	79.00 ^c (62.73)	16.38 ^d	5.48 ^e	1627.40 ^e	74.00 ^c (59.00)	15.88 ^f	5.38 ^d	1573.24 ^f

*Mean of three replications. Data in parenthesis are angular transformed values. In a column, mean followed by a common letter are not significantly different at 5 % level by DMRT.

Propiconazole (0.1%), Carbendazim (0.1%) + Mancozeb (0.1%) treatments from both trial I and II recorded statistically significant maximum seed germination (>90 %) and seedling vigour after six months of storage. This may be due to the enhanced fluorescent pseudomonad mediated disease resistance in rice plants or prevalence of minimum frequency of mycoflora at the time of harvest and inactivation or killing of fungi in the seed individually by the residues of *P. fluorescens* and fungicides even during storage. Earlier reports also envisaged that *P. fluorescens* (Velusamy *et al.*, 2013) and propiconazole (Lore *et al.*, 2007) improved seed germination (%) of rice by eradicating the seed mycoflora.

4. Conclusion

The results of the present study clearly showed that foliar spray of biocontrol agents, botanicals and fungicides has its own significance in controlling diseases and frequency of mycoflora in the seed. The foliar spraying with either *P. fluorescens* + butter milk (0.5%) or *P. fluorescens* (0.5 %) has reduced incidence of sheath rot, false smut and grain discolouration diseases, seed mycoflora load and sustained maximum germination after six months of storage under gunny bags in the ambient storage and hence suggested as suitable strategy to manage diseases of rice during *rabi* and to produce healthy seed in the coastal areas.

Conflict of Interest

Authors declare that they have no conflict of interest.

Ethical Compliance Statement

NA

Author's Contribution

CJ, RR: Collection of literature, Conceptualization, Compilation, Writing original draft; TR: Final editing, Proof Reading

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