# Response of Different Bio-control Agents and Fungicides against Pathogen Associated with Rice (*Oryza sativa* L.) Seedling Blight Disease under Nursery Conditions

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Abstract: Rice seedling blight in nurseries is more severe in rice that has been seeded early when the soil is usually cold and damp conditions that tend to delay seedling emergence favour seedling blight. Blighted seedlings emerge from the soil and die soon after emergence. Seedlings generally survive a lack of vigor with yellow to pale green colour and do not compete well with healthy seedlings. The object of this study was to identify pathogens associated with rice seedling blight disease and its management in nurseries condition. Three Pathogens, viz., Sclerotium rolfsii, Fusarium moniliformae and Curvularia lunata were found associated with rice seedling blight disease that occurred in nurseries raised for summer rice cultivation. Four biocontrol agents and seven fungicides were evaluated under invitro and invivo conditions. Among the bio agents, maximum mycelial growth inhibition (56.11%) of S. rolfsii by Trichoderma viride was recorded. Maximum mycelial growth of Fusarium moniliformae inhibited by T. harzianum with 70.56 per cent. While, maximum mycelial growth of *Curvularia lunata* inhibited (71.94%) by *T. harzianum*. Moreover T. harzianum and T. viride were found most superior over the other bio-agents tested under in vitro conditions. In case of fungicide, seven were tested against S. rolfsii, Fusarium moniliformae and Curvularia lunata to inhibit their mycelium growth. Among the seven fungicides, pyraclostrobin 133g/1 + epoxiconazole 50g/l) and tebuconazole 25% EC) at 100 ppm concentration completely inhibit the mycelia growth of fungi. In nursery conditions rice seeds treated with Pseudomonas fluorescens 1x10<sup>8</sup> cfu/ ml @ 10 ml/kg seeds and spray with azoxystrobin 23% EC at 0.05% at 15 DAS and seeds treated with azoxystrobin 23% EC at 0.05% solution gave highest seed germination with minimum seedling mortality up to 21 days after sowing.

Keywords: Seed germination, seedling blight, fungicides, bio-control root and shoot

## 1. INTRODUCTION

Rice (*Oryza sativa* L.) is one of the most important food-grain in the world wide, accounting for more than 20 per cent of global calories consumed. Rice is the second largest produced cereal in the world. India is one of the world's largest producers of rice, it occupies an area of 43.79 MHa, production of 116.42MT and productivity of 2.659 tones/ha (Anon., 2019b). In Gujarat it occupies an area of 8.38 lakh ha, production of 1.91MT and productivity of 2278 kg/ha (Anon., 2019a). Rice crop affected by so many diseases such as seedling blight, blast, sheath blight, sheath rot, brown spot, false smut, water-mold and seed-rot and bacterial leaf blight have been reported from many rice growing areas of India. Among that seedling blight or damping off is an important disease complex caused by several seed borne and soil borne fungi *e.i.*, *Cochiobolus* sp., *Curvularia sp., Sarocladium sp., Fusarium sp., Rhizoctonia sp.,* and *Sclerotium sp.* (Groth, 1991). Rice seedling blight disease is caused by *Sclerotium rolfsii* in the rice nursery of the boro season (Shahjahan *et al.,* 1987). Typically, the rice seedlings are weakened or died due to the fungi. Cold and wet weather environmental conditions favourable for disease development (Groth, 1991). Seedling blight is more severe in rice that has been seeded early when the soil is usually cold and damp. Conditions that tend to delay seedling emergence favour seedling blight. Seedling blight of rice affected at the time of germination can be reduced by treating the seed with fungicides. The main objective of the present study was to identifying associated organism caused seedling blight disease in rice and it's management under nurseries conditions.

### 2. MATERIAL AND METHODS

### 2.1. *Invitro* evaluation of bio-agent against fungal pathogens associated with rice seedling blight disease

Fungal pathogens associated with rice seedling blight were isolated from infected rice seedling samples which were collected from rice nurseries of Regional Rice Research Station, NAU, Vyara. Three dominant fungal pathogen viz., S. rolfsii, Fusarium moniliformae and Curvularia lunata were isolated on PDA medium and maintained at 27 ± 2°C on potato dextrose agar media for further studies. Four bio-agents i.e., Trichoderma viride, Trichoderma harzianum, Pseudomonas fluorescens and *Bacillus subtillis* were brought from Department of Plant Pathology, N. M. College of Agriculture, Navsari Agricultural University, Navsari. Invitro experiment was conducted using completely randomized block (CRD) design with 5 treatments and each repeated four times.

The test bio-agent and the pathogens were grown on PDA and from 10 days old culture, a 5 mm disc of the test organism (antagonist) was cut aseptically from the periphery of the colony and placed at one end of the Petri plate containing 20 ml PDA media. In the opposite place and approximately 60 mm away from the first, a similar disc of the pathogen was aseptically placed. Repetitions of each were kept and the plates with only pathogen served as control. The plates incubated in BOD incubator at 25  $\pm$ 2°C and the radial growth of the test organism and pathogen was measured after control plate covered completely with mycelium of the pathogen. The per cent growth inhibition (PGI) was calculated as per formula given by Vincent (1947).

$$PGI = \frac{DC - DT}{DC} \times 100$$

Where;

PGI = Percent growth inhibition

DC = Average diameter of mycelial colony (mm) control

DT = Average diameter of mycelial colony (mm) treated

### 2.2. *In vitro* evaluation of fungicides against fungal pathogens associated with rice seedling blight disease

Efficacy of different fungicides was studied under in vitro condition following by "poisoned food technique" against different isolates (Nene and Thapliyal, 1993). The experiment was conducted using completely randomized block (CRD) design with 8 treatments and each repeated three times. Seven different fungicides were used for study at three concentrations viz., 100 ppm. Required quantity of each fungicide under study was mixed thoroughly in sterilized 100 ml PDA media filled in 250 ml flask separately under aseptic condition. The medium was supplemented with few pinch of streptomycin sulphate to prevent bacterial contamination. The poisoned medium was then poured in sterilized Petri plates (20 ml) and allowed it to solidify. The plates were then inoculated with five mm diameter disc of seven days old culture of test pathogen by placing in the center of the plate. Control was maintained for each set where fungal disc was placed on PDA medium without fungicide. The inoculated plates were then incubated at 25 ± 2°C in BOD incubator. The observations were recorded for radial growth in mm after full growth in control Petri plate. Per cent mycelial growth inhibition was measured after the colony in the control plate was covered with mycelium of pathogen. The per cent growth inhibition (PGI) was calculated as per formula given by Vincent (1947).

$$PGI = \frac{DC - DT}{DC} \times 100$$

Where;

PGI = Percent growth inhibition

DC = Average diameter of mycelial colony (mm) control

DT = Average diameter of mycelial colony (mm) treated

# 2.3. Field evaluation of bio-agents and fungicides under nursery conditions

Field experiment was conducted to study the efficacy of selective fungicides and bio-agents used as seed treatment for the management of seedling blight disease caused by S. rolfsii, Fusarium moniliformae and Curvularia lunata. Treatments details are as, T<sub>1</sub>:seeds treated with Trichoderma viride (2x10<sup>6</sup> cfu/g) @ 10g/kg; T<sub>2</sub>: seeds treated with Trichoderma harzianum  $(2x10^6 \text{ cfu/g}) \otimes 10 \text{g/kg}; \text{T}_3$ : seed treated with Pseudomonas fluorescens (1x10<sup>8</sup> cfu/ml) @ 10ml/ kg;  $T_{4}$ : seeds treated with with *Trichoderma* viride (2x10<sup>6</sup> cfu/g) @ 10 g/ kg and sprayed with pyraclostrobin133g/l+ epoxiconazole 50g/l at 0.05% at 15 DAS; T<sub>5</sub>: seeds treated with Trichoderma harzianum (2x10<sup>6</sup> cfu/g) @ 10 g/ kg and sprayed with tebuconazole 25% EC at 0.05% at 15 DAS; T<sub>6</sub>: seeds treated with *Pseudomonas* fluorescens (1x108 cfu/ml) @ 10 ml/ kg and sprayed with azoxystrobin 23% EC at 0.05% at 15 DAS;  $T_{\tau}$ : seeds soaked with pyraclostrobin133g/ 1+ epoxiconazole 50g/1 at 0.05 % solution for 3 hrs; T<sub>s</sub>: seeds soaked with szoxystrobin 23% EC at 0.05 % solution for 3 hrs;  $T_0$ : seeds soaked with tebuconazole 25% EC at 0.05 % solution for 3 hrs;  $T_{10}$ : check.

The field experiment was arranged in randomized block design with ten treatments and three replications during *Summer* 2020-21 at Regional Rice Research Station, Navsari Agricultural University, Vyara. In raised bed nurseries, rice variety GNR-3 was sown by broadcasting method in 1.1m<sup>2</sup> plots sized. 2 kg FYM in each plot and half dose of ammonium sulphate applied at the time of sowing. 25 g ammonium sulphate applied in two equally spit dose at 15 days intervals.

Observations were recorded at 21 DAS. Germination per cent and seedling mortality were recorded.

$$Germination(\%) = \frac{Number of germinated seeds}{Total number of seed sown} \times 100$$

Seedling mortality was calculated by the following formula

$$Seedling mortality(\%) = \frac{Initial seedling population -}{Final seedling population} \times 100$$

#### 3. RESULTS AND DISCUSSION

# 3.1. *In vitro* evaluation of bio-agent against test pathogens

The results described in table-1 and fig.-1 revealed that all the treatments significantly reduced the mycelial growth of all the pathogens over the control.

3.1.1. Inhibition of S. rolfsii: Maximum mycelial growth inhibition (56.11%) was observed in treatment  $T_1$  (*T. viride*) at 4 days after incubation followed by treatment  $T_{4}$  (B. subtilis) and  $T_2$  (T. harzianum) by 44.72 and 39.45 per cent, respectively. Least mycelial growth inhibition (28.06%) was recorded in treatment  $T_3$ (*P. fluorescens*). *T. viride* was found most superior to over the other bioagents tested under *in vitro* condition (fig.1 A). The present results are more or less in agreements with the earlier workers reported by Muhammad and Amusa (2003) tested Bacillus subtilis against Sclerotium rolfsii under in *vitro* condition by the dual culture technique and reveled that Bacillus subtilis induced inhibition of growth by 44.4 percent. Similarly reported by Bhuiyan et al. (2012) T. harzianum reduced more than 60 percent mycelial growth inhibition of S. rolfsii over the control. T. viride isolates, T<sub>1</sub> (showed higher growth inhibition 75.3 per cent of *S. oryzae* and among *P. fluorescens* isolates, BI-1 showed maximum (77.2%) growth inhibition (Gopika et al.2016). T. harzianum and T. viride mycelial growth inhibition of S. oryzae by 40.11 and 39.59 per cent, respectively (Kumar et al., 2018). Swain *et al.* (2018) assessed ability of the six Trichoderma isolates against S. rolfsii and found 80.25–100.00 per cent inhibition by different isolates.

**3.1.2. Inhibition of** *Fusarium moniliformae*: Maximum mycelial growth inhibition was

observed in treatment T<sub>2</sub> (T. harzianum) by 70.56 per cent which was at par with treatment  $T_1$  (*T*. viride) at 69.45 percent at 7 days after incubation. Least mycelial growth inhibition (39.17%) was recorded in  $T_4$  (*P. fluorescens*). *T. harzianum* and *T. viride* were found most superior over the other bioagents tested under *in vitro* condition(fig.1 B). The present results are more or less in agreements with the earlier workers reported by Muhammad and Amusa (2003) tested Bacillus subtilis against Fusarium oxysporum under in vitro condition by the dual culture technique and reveled that *Bacillus subtilis* induced inhibition of growth by 75.5 percent. Verma et al. (2018) reported 85.7 percent mycelial growth inhibition of F. oxysporum was observed by Pseudomonas sp. (strain SY1). Similarly, Deb and Khair (2018) reported that T. harzianum inhibited growth of *Fusarium moniliforme* by 65.03 per cent.

3.1.3. Inhibition of *Curvularia lunata*: Maximum mycelial growth inhibition (71.94%) was observed in treatment  $T_2$  (*T. harzianum*) at 7 days after incubation followed by treatment T (B. subtilis) and  $T_1$  (T. viride) with 58.34 and 58.33 per cent, respectively. Least mycelial growth inhibition (55.00%) was recorded in treatment T<sub>3</sub> (P. fluorescens). T. harzianum was found most superior to over the other bioagents tested under *in vitro* condition (fig.1 C). The present results are more or less in agreements with the earlier workers reported by Verma et al. (2018) reported 86.3 per cent mycelium growth inhibition of Curvularia sp. was observed by Pseudomonas sp. (strain SY1) Deb and Khair (2018) reveled that T. harzianum inhibited growth of Curvularia lunata by 36.35 per cent.

# 3.2. *In vitro* evaluation of fungicides against test pathogens

The observations regarding per cent inhibition of mycelial growth are presented in table-2 revealed that all the fungicides were significantly inhibit the growth of the pathogens.

**3.2.1. Inhibition of** *Sclerotium rolfsii*: The result revealed that all the fungicides significantly inhibited the mycelial growth of *S. rolfsii*. Cent per cent mycelial growth inhibition was recorded in treatment  $T_4$  (pyraclostrobin 133g/l+ epoxiconazole 50g/l) and  $T_5$  (tebuconazole 25%)

EC) at all the tested concentrations and also found most effective in inhibiting the mycelial growth at 100 ppm concentration over control followed by treatment  $T_2$  (azoxystrobin 23% SC) with 82.59 per cent growth inhibition (table 2). While treatment  $T_3$  (azoxystrobin 11% + tebuconazole 18.3%) was found least effective as compared to other fungicides with 59.99 per cent mycelial growth inhibition (fig.2 A). The present results are more or less in agreements with the earlier workers reported by Khan and Javaid (2015) tested four different fungicides against S. rolfsii at 50, 100, 150, 200 and 250 ppm concentrations among them tebuconazole completely inhibited growth of fungi at all concentrations. Gopika *et al.* (2016) evaluated six fungicides against *Sclerotium* oryzae Catt and found that hexaconazole 5 EC at 200 ppm and propiconazole 25 EC at 100 ppm completely inhibited S. oryzae by using poisoned food technique. Soyal and Ratnoo (2018) revealed that S. rolfsii mycelial growth inhibition was recorded in tebuconazole 25.9 EC by 89.26 per cent at 500 ppm and 93.71 per cent at 750 ppm by hexaconazole 5 EC. Rangarani et al. (2018) evaluated fifteen fungicides at three different concentrations against S. oryzae found that propiconazole 25 EC at 500 ppm, hexaconazole 5 EC 1000 ppm, tebuconazole 25.9 EC 750 ppm, trifloxystrobin + tebuconazole (75 WG) 400 ppm, Azoxystrobin 23 SC 500ppm completely inhibited the growth of *S. oryzae*. Prameela *et al*. (2018) tested eight fungicides against S. oryzae by using poisoned food technique and revealed that propiconazole 25 EC at 100 ppm and hexaconazole 5 EC at 200 ppm showed complete inhibition growth followed by tebuconazole 60 FS (97.77%).

**3.2.2. Inhibition of** *Fusarium moniliformae*: A significantly mycelial growth inhibition of was observed in all the fungicides under *in vitro* condition (table 2 and fig. 2 B). Completely mycelial growth inhibition was recorded in treatment  $T_4$  (pyraclostrobin 133g/ 1+ epoxiconazole 50g/l) and  $T_5$  (tebuconazole 25% EC) (table 2) followed by treatment  $T_1$ (trifloxystrobin 25% + tebuconazole 50% WG) at 100 ppm concentration. While treatment  $T_6$ (propiconazole 25% EC) and  $T_7$  (hexaconazole 70% WP) were found least effective at all concentrations as compared to other fungicides with 64.81 and 65.92 per cent, 67.04 at 100 ppm concentration, respectively. Our findings more or less similar with Bhali *et al.* (2001) observed that propiconazole at 100 ppm concentration inhibit the mycelial growth by 81.82 per cent. Similar finding by Jain et al. (2014) reported that hexaconazole and tebuconazole completely inhibited the growth of *Fusarium monoliformae* at 100 ppm. Chowdhury et al. (2015) observed that propiconazole showed completely growth inhibition of *F. moniliformae* and hexaconazole at 500 ppm inhibited 65.5 percent mycelial growth. Propiconazole at 0.1 per cent could completely inhibit the growth of *F. moniliformae* (Salma *et al.*, 2015).

3.2.3. Inhibition of Curvularia lunata: Cent per cent mycelial growth inhibition was recorded in treatmet  $T_4$  (pyraclostrobin 133g/l+ epoxiconazole 50g/l) and  $T_5$  (tebuconazole 25%) EC) at all the tested concentrations and also found most effective in inhibiting the mycelial growth at 100 ppm concentration over control followed by treatment T<sub>2</sub> (azoxystrobin 23% SC). While treatment  $T_1$  (trifloxystrobin 25% + tebuconazole 50% WG) was found least effective as compared to other fungicides with 56.67 per cent mycelial growth inhibition(table 2 and fig.2 C). Present results coherence with Chowdhury *et al.* (2015) observed that propiconazole showed completely growth inhibition of C. lunata and hexaconazole at 500 ppm inhibited 80 percent mycelial growth.

# 3.3. Evaluation of bio-agents and fungicides under nursery condition

The experiments results presented in table-3 revealed that all the treatments were found significantly superior seed to improve germination as compared to check. Highest seed germination (71.39%) was observed in treatment  $T_{8}$  (seeds treated with azoxystrobin 23% SC at 0.05 % conc.) which was at par with treatment  $T_{6}$  (seeds treated with *Pseudomonas fluorescens* and spray with azoxystrobin 23% SC) and  $T_{a}$ (seeds treated with tebuconazole 25.9% EC) by 67.77 and 67.35 per cent, respectively. Whereas minimum seed germination was observed in treatment  $T_{10}$  (control) with 58.99 per cent. Moreover seeds treated with azoxystrobin 23%

SC at 0.05 percent solution or seeds treated with *Pseudomonas fluorescens* and spray with azoxystrobin 23% SC at 0.5% at 15DAS or seeds treated with tebuconazole 25.9% EC found best treatment to improved seed germination. Our findings are similar to early workers by Amein *et al.* (2008) wheat seeds treated with *P. fluorescens* Strains 1 and 3 has significantly increased seedling emergence of wheat by 15 and 17 per cent, respectively. Similarly reported by Sengupta *et al.* (2015) maize seed treatment with PGPR strains of *Pseudomonas* sp. and *Azotobacter* improved seed germination, seedling vigour, seedling emergence and seedling stand over the control.

Seedling mortality results presented in table 3 recorded that all the treatments were found significantly superior over the check. Minimum per cent seedling mortality by 2.60 percent was observed in treatment T<sub>6</sub> (ST with *Pseudomonas fluorescens* 1x10<sup>8</sup> cfu/ml @ 10 g/ kg seeds and spray with azoxystrobin 23% EC at 0.05% at 15 DAS which was at par with  $T_5$  (seeds treated with Trichoderma harzianum @ 10 g/ kg and sprayed with tebuconazole 25% EC at 15 DAS),  $T_{A}$  (seeds treated with with *Trichoderma viride* @ 10 g/ kg and sprayed with pyraclostrobin133g/ 1+ epoxiconazole 50g/l at 0.05% at 15 DAS) and  $T_{s}$  (seeds soaked with szoxystrobin 23% EC at 0.05 % solution for 3 hrs) with 2.69, 3.20 and 3.25 per cent, respectively. Seedling mortality when seeds treated with T. harzianum, P. fluorescens and *T. viride* were showed by 3.87, 3.98 and 5.39 per cent, respectively. While least effect on seedling mortality by treatment  $T_{o}$  (seeds treated with tebuconazole) with 10.37 per cent. Maximum seedling mortality (16.98%) was observed in treatment  $T_{10}$  (check). Moreover all treatments were minimized seedling mortality as compared to check. Our results are more or less agreements with earlier workers, Elad *et al.* (1982) reported that soil application with *Trichoderma harzianum* (TH 294 and TH 203) significantly reduced P. aphanidermatum by isolate TH 294 and S. rolfsii disease was significantly reduced by isolate TH 203. Amein *et al.* (2008) wheat seeds treated with P. fluorescens Strains 1 and 3 reduced wheat seedling blight disease incidence in heavily infested winter wheat by 36 per cent. Plant

#### Table 1: Evaluation of different bio- agents against fungal pathogens associated with rice seedling blight under *in vitro* condition

	Name of biocontrol agent	Mycelial growth inhibition (%)			
Treat ment		S. rolfsii	F.moniliformae	C.lunata	
T <sub>1</sub>	Trichoderma viride Pers., NAU isolate	56.11(48.49)*	69.45(56.43)	58.33(49.78)	
Τ,	Trichoderma harzianum Rifai., NAU isolate	39.45(39.89)	70.56(57.11)	71.94(58.00)	
T <sub>3</sub>	Pseudomonas fluorescens Migula., NAU isolate	28.06(31.97)	39.17(38.73)	55.00(47.85)	
T <sub>4</sub>	Bacillus subtilis Ell., NAU isolate	44.72(41.95)	55.84(48.33)	58.34(49.78)	
T <sub>5</sub>	Control	00.00(0.00)	00.00(0.00)	00.00(00.00)	
SEm±		0.67	0.39	0.45	
CD at 5%		2.01	1.19	1.37	
CV %		3.95	2.42	2.24	

Average of four replications

\*Figures in the parenthesis are arc sine transformed value.

# Table 2: Effect of different fungicides on mycelial growth of Sclerotium rolfsii under in vitro condition

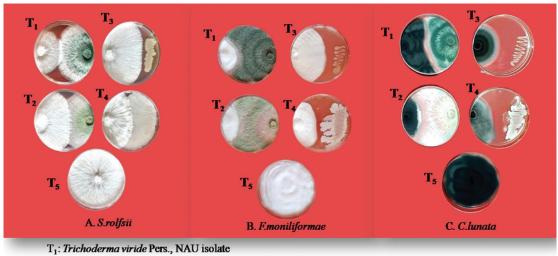
Treatment	Technical name of fungicide	<i>Mycelial growth</i> <i>Inhibition (%) at 100 ppm concentration</i>		
		S. rolfsii	F.moniliformae	C.lunata
T <sub>1</sub>	Trifloxystrobin (25%) + Tebuconazole (50%) WG	68.52(55.85)*	66.66(54.72)	56.67(48.81)
T <sub>2</sub>	Azoxystrobin 23% SC	82.59(65.33)	74.07(59.37)	92.22(73.80)
T <sub>3</sub>	Azoxystrobin 11% + Tebuconazole18.3%	59.99(50.75)	71.11(57.47)	60.74(51.18)
T <sub>4</sub>	Pyraclostrobin133g/l+ Epoxiconazole50 g/l	100.00(90.00)	100.00(90.00)	100.00(90.00)
T <sub>5</sub>	Tebuconazole 25% EC	100.00(90.00)	100.00(90.00)	100.00(90.00)
T <sub>6</sub>	Propiconazole 25% EC	63.70(52.94)	64.81(53.60)	71.85(57.94)
T <sub>7</sub>	Hexaconazole 70% WP	63.33(52.71)	65.92(54.27)	61.11(51.40)
T <sub>8</sub>	Control	0.00(0.00)	0.00(0.00)	0.00(0.00)
SEm±		0.44	0.48	0.40
CD at 5%		1.33	1.47	1.22
CV %		1.33	1.46	1.21

Average of three replications. \*Figures in the parentheses are arc sine transformed value

#### Table 3: Effect of different treatments of bio-agents and fungicides on rice seeds germination and seedling mortality

Treatment	Treatment Detail	Germination (%)	Mortality % at 21 DAS
T <sub>1</sub>	ST with Trichoderma viride	59.60 (50.54)*	5.39 (13.34)
T <sub>2</sub>	ST with Trichoderma harzianum	66.12 (54.41)	3.87 (11.34)
T <sub>3</sub>	ST with Pseudomonas fluorescens	63.62 (52.95)	3.98 (11.50)
T <sub>4</sub>	ST with <i>Trichoderma viride</i> and Spray with Pyraclostrobin + Epoxiconazole	59.22 (50.33)	3.20 (10.31)
T <sub>5</sub>	ST with <i>Trichoderma harzianum</i> and Spray with Tebuconazole	61.94 (51.91)	2.69 (9.41)
T <sub>6</sub>	ST with <i>Pseudomonas fluorescens</i> and Spray with Azoxystrobin	67.77 (55.43)	2.60 (9.24)
T <sub>7</sub>	ST with Pyraclostrobin + Epoxiconazole	59.14 (50.28)	4.84 (12.62)
T <sub>8</sub>	ST with Azoxystrobin	71.39 (57.73)	3.25 (10.37)
T <sub>9</sub>	ST with Tebuconazole	67.35 (55.24)	10.37 (18.76)
T <sub>10</sub>	Control	58.99 (50.19)	16.98 (24.32)
	SEm±	1.48	0.67
	CD at 5%	4.40	1.99
	CV %	4.85	8.83

Average of three replication. \*Figures in the parentheses are arc sine transformed value

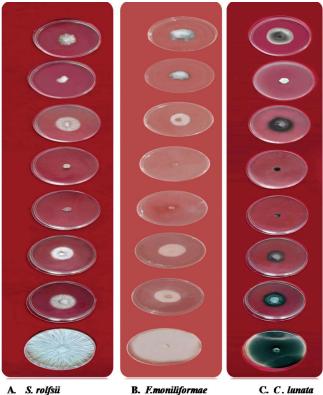


T2: Trichoderma harzianum Rifai., NAU isolate

- T3: Pseudomonas fluorescens Migula., NAU isolate
- T4: Bacillus subtilis Ell., NAU isolate

T5: Control

#### Figure 1: Evaluation of different bio-control agents against S. rolfsii, F.monliformoe and C.lunata under in vitro condition



A. S. rolfsü

C. C. lunata

- T1: Trifloxystrobin (25%) + Tebuconazole (50%) WG
- T2: Azoxystrobin 23% SC
- T<sub>3</sub>: Azoxystrobin 11% + Tebuconazole18.3%
- T4: Pyraclostrobin133g/l+Epoxiconazole50 g/l
- T<sub>5</sub>: Tebuconazole 25% EC
- T<sub>6</sub>: Propiconazole 25% EC
- T7: Hexaconazole 70% WP
- T8: Control

Figure 2: Effect of different fungicides on mycelial growth of fungal pathogens associated with rice seedling blight under in vitro condition

establishment was increased by 65, 48 and 15 per cent when seeds treated with *P. fluorescens* Strain 2, Strain 2 and Strain, respectively as compared to control by 5 per cent. Verma *et al.* (2018) observed that rice seedlings treated with SY1(*Pseudomonas* sp.) reduced infection of *Fusarium* by 18.75 per cent as compare to control by 51.50 per cent. Our findings also agreements with earlier workers like Kumar *et al.* (2003), Gopika *et al.* (2016), Gopika *et al.* (2017), Gowdar *et al.* (2018).

## 4. CONCLUSION

From above results conclude that *T. harzianum* and *T. viride* were found most superior over the other bio-agents tested under *in vitro* condition against *S. rolfsii*, *F. moniliformae* and *C. lunata*. In case of fungicides, pyraclostrobin 133g/l + epoxiconazole 50g/l) and tebuconazole 25% EC) at 100 ppm concentration completely inhibit the mycelia growth of *S. rolfsii*, *F. moniliformae* and *C. lunata* fungus. Rice seeds treated with *Pseudomonas fluorescens* 1x10<sup>8</sup> cfu/ml @ 10 g/ kg seeds and sprayed with azoxystrobin 23% EC (0.05% conc. at 15DAS) and seeds treated with azoxystrobin 23% EC( at 0.05%) gave highest seed germination, minimum seedling mortality up to 21 DAS.

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*Competing interest:* The authors declare that they have no competing of interest.

*Author's Contributions:* Patoliya Prashant Rajeshbhai and Kedar Nath, both the author's deign an experiment, prepared the materials and conducted experiment. Prashant analyzed data performed the statistical analysis and drafted the manuscript. Kedar Nath, edited previous versions of the manuscript. Both authors have read and approved the final manuscript.

Consent: Not applicable

*Ethical approval:* This article does not contain any studies on human being or animals by any of the authors

### Reference

- Amein, T.; Omer, Z.; and Welch, C. (2008). Application and evaluation of *Pseudomonas* strains for biocontrol of wheat seedling blight. *Crop Prot.*, **27**(3-5): 532– 536.
- Anonymous (2019a). *Krushi, Khedut kalyan ane Sahkar Vibhag*. Retrieved from https://dag.gujarat.gov. in/estimate-guj.htm. [Accessed on 18 July,2020]
- Anonymous (2019b). *Directorate of Economics and Statistics*. Retrieved: https://eands.dacnet.nic.in/. [Accessed on 01 Aug, 2020]
- Baki, A. A. A. and Anderson, J. D. (1973). Vigor determination in soybean seed by multiple criteria. *Crop Sci.*, **13** (6): 630-633.
- Bhuiyan, M. A. H. B.; Rahman, M. T. and Bhuiyan, K. A. (2012). *In vitro* screening of fungicides and antagonists against *Sclerotium rolfsii*. *Afri. J. of Biotech.*, **11** (82): 14822-14827.
- Chowdhury, P.; Bashar, M. A. and Shamsi, S. (2015). *In vitro* evaluation of fungicides and plant extracts against pathogenic fungi of two rice varieties. *Bangladesh J. Bot.*, **24** (2): 251-259.
- Deb, S. C. and Khair, A. (2018). Control of seed borne fungi of rice by *Aspergillus* and *Trichoderma*. *SSRG Int. J. of Agri. & Env. Sci.*, **5** (1): 34-39.
- Elad, Y.; Chet, I. and Henis, Y. (1982). Degradation of plant pathogenic fungi by *Trichoderma harzianum*. *Can. J. Microbiol.*, **28**: 719-725.
- Gopika, K. and Jagadeeshwar, R. (2017). Studies on disease incidence and efficacy of fungicides, herbicides and antagonists micro flora against stem rot of rice (*Sclerotium oryzae*) along with integrated management. *Agri. Update*, **12** (7): 2077-2087.
- Gopika, K.; Jagadeeshwar, R.; Krishna Rao, V. and Vijayalakshmi, K. (2016). Salient research findings on rice stem rot disease (*Sclerotium oryzae* Catt.) and its management. *Int. J. of Pl., Ani. and Env. Sci.,* **6** (1): 80-82.
- Gowdar, S. B.; Deepa, H.; Bindhu, K. G. and Hurali, S. (2018). Evaluations of fungicides against paddy stem rot. *Int. J. of Current Microbiol. and Applied Sci.*, 7: 4024-4027.
- Groth, D. E. (1991). Rice diseases and disorders in Louisiana. *LSU Agricultural Experiment Station Reports*. 668 p.
- Jain, S. K.; Khilari, K.; Ali, M. and Singh, R. (2014). Response of *Fusarium moniliformae*- the causal organism of bakane disease of rice against different fungicides. *J. Life Sci.*, **9**: 413-416.

- Khan, I. H. and Javaid, A. (2015). Chemical control of collar rot disease of chickpea. *Pak. J. Phytopath.*, **27** (1): 61-68.
- Kumar, A.; Singh, R. and Jalali, B. L. (2003). Management of stem rot of rice with resistance inducing chemicals and fungicides. *Ind. Phytopath.*, **56** (3): 266-269.
- Kumar, M.; Ashraf, S. and Dhakar, N. (2018). Evaluation of phytoextracts and bio-agents against stem rot disease of paddy caused by *Sclerotium oryzae* Catt. *J. of Pharmaco. and Phytochem.*, 7 (2): 309-311.
- Muhammad, S. and Amusa, N. A. (2003). *In-vitro* inhibition of growth of some seedling blight inducing pathogens by compost-inhabiting microbes. *African J. of Biotech.*, **2** (6): 161-164.
- Nene Y. L. and Thapliyal P. N. (1993). Fungicides in Plant disease Control (3rd ed.). Oxford IBH publishing corporation, New Delhi. pp. 331.
- Prameela, M.; Rajeswari, B. and Krishnaveni, D. (2018). Efficacy of fungicides and herbicides against *Sclerotium oryzae* Catt. incitant of stem rot disease in rice. *Int. J. of Curr. Microbio. and App. Sci.*, 7 (2): 1650-1653.
- Rangarani, A.; Rajan, C.P.; Devi, R.S.; Reddy, V.A. and Sudhakar, P. (2018). Evaluation of fungicides on *Sclerotium oryzae*, incitant of rice stem rot disease. *J. of Pharmaco. and Phytochem.*, 7 (4): 01-03.
- Salma, B.; Tombisana Devi, R. K. and Iboton Singh, I. (2015). Evaluation of fungicides, biocontrol agents and botanicals for management of damping- off in

cabbage seedlings caused by *Fursarium moniliforme* sheld. *J. of App. and Nat. Sci.*, **7** (1): 106–110.

- Sengupta, C; Bhosale, A. and Malusare, S. (2015). Effect of plant growth promoting rhizobacteria on seed germination and seedling development of *Zea mays*. *Int. J. of Res. in Adv. Tech.*, Special Issue National Conference "ACGT 2015": 32-40.
- Shahjahan AKM, Ahmed HU, Miah SA, Sharma NR, Rahman MM (1987). Efficacy of fungicides as seed and soil treatment in controlling seedling blight disease of rice. *Tropical Pest Management*, 33(3):211-213. https://doi.org/10.1080/09670878709371152
- Soyal, S. S. and Ratnoo, R. S. (2018). Bio-efficacy and management of stem rot (*Sclerotium rolfsii*) of groundnut with different fungicides and bioagents. *Int. J. of Chem.l Stud.*, 6 (5): 492-495.
- Swain, H.; Adak, T.; Mukherjee, A. K.; Mukherjee, P. K.; Bhattacharyya, P.; Behera, S. and Bagchi, T. B. (2018). Novel *Trichoderma* strains isolated from tree barks as potential bio-control agents and bio-fertilizers for direct seeded rice. *Microbio. Res.*, 214: 83–90.
- Verma, S. K.; Kingsley, K. L.; Bergen, M. S.; Kowalski, K. P. and White, J. F. (2018). Fungal disease prevention in seedlings of rice (*Oryza sativa*) and other grasses by growth-promoting seed-associated endophytic bacteria from invasive *Phragmites australis*. *Microorg.*, 6 (21): 1-13.
- Vincent, J. M. (1947). Distortion of fungal hyphae in presence of certain inhibitors. *Nature*, 159: 850.