Bio-agent based module for integrated management of sheath blight (*Rhizoctonia solani*) of rice

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ABSTRACT

Rice (*Oryza sativa* L.) is an important cereal crop consumed worldwide and sheath blight caused by *Rhizoctonia solani* Künh is one of the limiting factors for its sustainable production. The experiments were conducted during rainy seasons of 2013 and 2014 to evaluate the performance of various treatments comprising bio-formulation Pusa 5SD prepared from *Trichoderma viride* (IBSD T20) and *T. virens* (IARI P3), liquid formulation of *Bacillus subtilis* (IBSD), fungicides hexaconazole and carbendazim + thiram by various methods of applications (seed treatment and foliar spray) to develop an effective module for the integrated management of the disease. Twenty treatments consisting of four levels of seed treatments and five levels of foliar spray including control for each factor were evaluated. A combination of seed treatment and foliar spray with *T. virens* (IARI P3) based Pusa 5SD provided the highest number of hills, tillers, plant height, number of grains, plant dry weight and grain yield along with the lowest relative lesion height and disease index. Whereas, the highest seed germination was recorded in a combination of seed treatment and foliar spray of *T. viride* (IBSD T20) based Pusa 5SD. A combination of seed treatment with *T. virens* (Pusa 5SD) and foliar spray of liquid formulation of *B. subtilis* (IBSD) was the next effective treatment for increasing the grain yield and yield attributing parameters and decreasing the disease. The module developed in the present study is suitable for management of the disease.

Key words: Bacillus subtilis, Integrated management, Rice, Sheath blight, Trichoderma viride, T. virens

Rice (*Oryza sativa* L.) is one of the most important cereal crops cultivated and consumed globally, mainly in Asian and developing countries. The continuous growth in rice consumption has imposed pressure on the production for developing sustainable crop cultivation techniques. Sheath blight caused by *Rhizoctonia solani* is one of the most destructive rice diseases worldwide, accounting for losses of 6–30% annually (Soe and De Costa 2012, Guo and Liao 2014, Ghosh *et al.* 2018) and considered as one of the most limiting factors to a sustainable rice production.

The sclerotia of the pathogen present in the paddy field germinate and produce mycelia, which colonise the lower leaf sheath, and produced necrotic lesions lead to high disease severity. The mycelia of the pathogen in infected rice plants and sclerotia present in the soil are the primary sources of inoculum (Srinivasachary *et al.* 2011). Sclerotia of *R. solani* can survive for years in the soil or on plant matter.

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The widespread cultivation of new, susceptible, highyielding cultivars with large numbers of tillers, and the changes in cultural practices associated with these cultivars, favour the development of sheath blight and contribute greatly to the rapid increase in the incidence and severity of the disease in rice-producing areas throughout the world (Groth et al. 1991). Furthermore, environmental conditions such as low light, cloudy days, high temperature and high relative humidity also favour the disease development. Control of the pathogen is difficult because of its ecological behavior, its extremely broad host range and the high survival rate of sclerotia under various environmental conditions (Groth and Bond 2006). So far, no rice variety completely resistant to the disease has been found, although extensive evaluation of rice germplasm has been conducted (Oard et al. 2004). In the absence of a desired level of host resistance, the disease is currently managed by excessive application of fungicides, which have adverse effects on the soil biota, pollute the atmosphere, and harmful to environment (Dass 1990). It is difficult to achieve desired level of control through host resistance or fungicides, therefore, biological control may be effective in minimizing the incidence of sheath blight (Das and Hazarika 2000).

Pre-application of the biocontrol agents allow the microorganisms to colonise the niche before the pathogens, and application of organic amendments increases the efficacy of biocontrol agents (Boukaew *et al.* 2013). Thus, the antagonists are mainly applied by spraying on the surface of the rice tissues or by soaking the rice seed or seedlings in a suspension of the biocontrol products before transplanting to the field (Soe and De Costa 2012). Successful biocontrol of sheath blight depends upon the ability to deliver the biocontrol agent to the sites where the pathogen is present (Yang *et al.* 2011, Wiwattanapatapee *et al.* 2013), the capacity of the substrate to maintain the vitality of the biocontrol agents (Schisler *et al.* 2004) and the population of the biocontrol agents (Harman *et al.* 2004).

The genus *Trichoderma* is especially known for its antagonistic activity against several plant pathogens, including *R. solani* (Dubey and Patel 2001, Harman 2006). The efficacy of the *Trichoderma* strains varied from place to place and its efficacy inhanced when applied with compatible chemicals and other bioagents. Methods and time of application of bio-agents matter for the management of such disease which is primary soil and seed borne and secondary spread by contact and air borne basidiospores. Thus, the management depends on the efficacy of bio-agents and method of application in time. Therefore, the aim of the present investigation was to develop an efficient biocontrol agent(s) based module for integrated management of sheath blight of rice.

MATERIALS AND METHODS

The experiments were conducted during rainy seasons of 2013 and 2014 in completely randomized design-factorial with three replications to evaluate the performance of various treatments comprising bio-formulation Pusa 5SD developed from Trichoderma viride and T. virens, liquid formulation of Bacillus subtilis, fungicides hexaconazole and carbendazim + thiram by various methods of application (seed treatment and foliar spray) under sick pot soil condition. Twenty treatments consisting of four levels of seed treatments (ST1, ST2, ST3 and ST4) as first factor and five levels of foliar spray (F1, F2, F3, F4 and F5) as second factor including controls for each factor were evaluated. Surface sterilized plastic pots (35 cm dia) were filled with sterilized (formalin 1%) soil (15 kg/pot) and inoculated with the inoculum of R. solani at 5 g/kg of soil at 2 days before sowing. The pathogen was multiplied on sorghum grains. The grains were soaked in tap water for 12 h, strained and filled into 500 ml conical flasks (250 g/flask). The flasks containing grains were autoclaved for 2 subsequent days at 1.1 kg/cm² for 30 min, inoculated with a 5-days old culture of R. solani and incubated for 15 days at 25±1°C. Bio-formulation Pusa 5SD developed following the procedures described earlier (Dubey et al. 2009) from T. viride (IBSD T20, Imphal) and T. virens (IARI P3, New Delhi) were used for seed treatment and foliar spray. Liquid formulation of Bacillus subtilis of IBSD, Imphal was also used for foliar spray. The seeds were treated with bioformulation (Pusa 5SD) at 4 g/ kg of seed while, carbendazim + thiram was used at 2 g/kg (1:1 ratio). Untreated seeds were maintained as a control. Twenty seeds/pot of susceptible rice variety Pusa Basmati

1509 were sown on July 26, 2013 and July 28, 2014. The plants were inoculated by placing 3 pieces of well colonized typha stem (5 cm) with *R. solani* at boot stage on September 23, 2013 and September 30, 2014. Twenty such stem pieces were placed in 250 ml conical flask and autoclaved at 1.1 kg/cm² for 30 min. The flask containing typha stem pieces were inoculated with 4 days old culture of *R. solani* and incubated for 7 days. The plants were sprayed with 5 ml/litre liquid formulation of *B. subtilis* whereas, Pusa 5SD of *T. virens* and *T. viride* at 5 g/litre and hexaconazole at 1 ml/litre at 4 days after aerial inoculation. The plants with water spray were maintained as control. Proper humidity was maintained for disease development by spraying water regularly.

Germination was recorded 15 days after sowing. Observations were recorded for number of hills and tillers per pot before aerial inoculation. Plant height, sheath height, lesion size were recorded by taking 2 tillers/hill and 5 hills/ pot at 75 days after sowing. Number of grains/ear was recorded by selecting 5 tillers from each pot randomly before harvesting of the crop. Dry weight of plants and grain yield/ pot were recorded. Relative lesion height (RLH) and percent disease incidence (PDI) were calculated by using standard evaluation system for rice, Philippines: International Rice Research Institute. The RLH was calculated by the formula as lesion size/sheath height x 100 and PDI was calculated by randomly selected 2 plants/hill and 5 hills/pot using 0-9 grade scale as sum of all diseased rating x 100/number of plants observed x maximum grade (IRRI 2002). The data pertaining to all the observations were subjected to ANOVA (Gomez and Gomez 1984) using the SAS Software (SAS Institute, version 9.1, Cary, NC). The data were analyzed as per the procedure for a completely randomized designfactorial for the test of significance. Fisher's Protected Least Significant Differences (LSD) was computed only when ANOVA showed significant differences for any particular effect.

RESULTS AND DISCUSSION

Effect of seed treatment

Except carbendazim + thiram treated seeds for seed germination, and number of hills/pot for 2013 and number of tillers/hills and number of grains per ear for 2014, all the treatments proved significantly superior over the control for all the recorded variables. Seed treatment with IARI, New Delhi isolate of *T. virens* based Pusa 5SD (ST2) provided the highest seed germination, number of hills, tillers, plant height, number of grains, plant dry weight and grain yield and the lowest relative lesion height and disease index during both the years of experimentations. The next effective treatment was seed treatment with IBSD, Imphal isolate of T. viride based Pusa 5SD (ST1) for all the variables recorded. The seed germination, number of hills and tillers/pot recorded in these two treatments did not differ significantly in 2013 and in 2014, seed germination, number of tillers and plant height, recorded in these two treatments did not differ

significantly. Fungicide (carbendazim + thiram) ranked third in the performance for all the variables (Table 1 and 2).

A seed dressing formulation Pusa 5SD prepared from a potential isolate of T. virens (IARI P3) was proved highly effective for seed treatment as well as foliar spray (Dubey et al. 2011; Dubey et al. 2012). Seeds treated with Pusa 5 SD provided the highest grain yield and supported maximum for yield attributing parameters like hills, tillers, plant height, grain numbers and dry plant weight along with the lowest development of sheath blight in terms of relative lesion height and disease index. Similar formulation prepared from IBSD, Imphal isolate of *T. viride* also proved next effective treatment. Chen et al. (2015) used rice hull carrier based formulation of T. asperelleum and obtained about 90 % of bio-control efficacy and significantly increased the grain weight of rice as compared to control. The present findings are supported by the observations of Da Silva et al. (2012) that *Trichoderma* application as seed treatment significantly reduced the sheath blight. Shekhada et al. (2018) reported the use of *T. harzianum* for seed bio-priming as an excellent bio-agent for the management of sheath blight.

Effect of foliar spray

Except foliar spray of hexaclonazole for germination in 2013 and for number of tillers and plant height in 2014, all other spray treatments significantly increased the hills per pot, number of tillers per hill, plant height, dry weight of plant, number of grains per ear and grain yield per pot and reduced the relative lesion height and disease index over the control. Except seed germination for 2014,

the spraying of Pusa 5SD of *T. virens* (F2) provided the highest yield and yield attributing parameters with lowest disease development. Its performance was statistically similar with Pusa 5SD formulation of *T. viride* (F1) for germination, number of hills per pot, tillers per hill, grains per ear whereas, for seed germination, hills per pot, tillers per hill, plant height, relative legion height and grains per ear it was similar with *B. subtilis* (F3) during 2013 (Table 3). The grain yield recorded in *T. viride* and *B. subtilis* did not differ significantly during 2014. Number of hills, tillers, plant height, and number of grains, plant dry weight and grain yield recorded in spray of *T. virense* (Pusa 5SD) and *B. subtilis* during 2014 did not differ significantly (Table 4).

Effect of interactions of seed treatment and foliar sprays

Among the interactions, a combination of seed treatment and foliar spray with *T. virens* (IARI) of Pusa 5SD (ST2×F2) provided the highest plant height, number of grains per ear, dry plant weight and grain yield with the lowest relative lesion height and disease index during 2013 (Table 5) and during 2014 (Table 6), similar interaction provided the highest number of hills, tillers, plant height, grain number, plant dry weight and grain yield along with the lowest relative lesion height and disease index, while the highest seed germination was recorded in a combination of seed treatment with Pusa 5SD (*T. viride*) and foliar spray with *T. viride* (ST1×F1). A combination of seed treatment with Pusa 5SD (*T. virens*) and foliar spray of *B. subtilis* (ST2×F3) was the next effective treatment for increasing the yield and yield attributing parameters and decreasing

Table 1 Effect of different seed treatments on germination, plant height, number of hills and tillers, plant dry weight, grain yield, relative lesion size and disease index in rice during 2013

Treatment	Details	Germination	Hills	Tillers	Plant	Relative	Disease	Grains	Dry	Grain
		(%)	(no./	(no./	height	lesion height	index	(no./	weight	yield
			pot)	hill)	(cm)	(%)	(%)	ear)	(g/plant)	(g/pot)
ST1	Pusa 5SD	92.3 (74.7) ^{ab}	16.3a	10.5a	65.0 ^b	20.7 (26.7) ^b	23.4 (28.4) ^b	57.0 ^b	11.0 ^b	30.4 ^b
	(T. viride -IBSD T20)									
ST2	Pusa 5SD	93.7 (77.3) ^a	17.2a	11.1 ^a	67.8a	17.1 (24.1) ^a	18.4 (24.8) ^a	60.7a	13.6a	36.5a
	(T. virens -IARI P3)									
ST3	Carbendazim +Thiram	90.3 (72.1)bc	16.3ab	9.7^{b}	63.1 ^c	21.1 (27.3) ^c	25.2 (30.0) ^c	55.8 ^b	9.5°	28.2 ^c
ST4	Control (no treatment)	87.0 (69.3) ^c	15.5 ^b	7.9 ^c	58.1 ^d	31.5 (33.9) ^d	41.2 (39.7) ^d	48.7 ^c	8.3 ^d	24.5 ^d

Figures in parentheses are transformed angular values. The values within a column with different letters are significantly different at 5% level by using Fisher's least significance difference test.

Table 2 Effect of different seed treatments on germination, plant height, number of hills and tillers, plant dry weight, grain yield, relative lesion size and disease index in rice during 2014

Treatment	Details	Germination (%)	Hills (no./	Tillers (no./ hill)	Plant height	Relative lesion height	Disease index (%)	Grains (no./	Dry weight	Grain yield
			pot)		(cm)	(%)		ear)	(g/plant)	(g/pot)
ST1	Pusa 5SD	72.3 (59.9) ^a	12.7 ^b	3.9 ^{ab}	74.9 ^{ab}	19.1 (25.7) ^b	20.9 (26.9) ^b	57.1 ^b	22.5 ^b	47.5 ^b
	(T. viride -IBSD T20)									
ST2	Pusa 5SD	73.3 (59.6) ^a	15.2a	4.4^{a}	76.9a	16.1 (23.3) ^a	17.3 (24.3) ^a	60.8a	32.5a	52.7a
	(T. virens -IARI P3)	,				, ,				
ST3	Carbendazim +Thiram	67.0 (55.3) ^b	12.6 ^b	3.6bc	74.1 ^b	21.5 (27.6) ^c	22.3 (28.1) ^c	55.2 ^{bc}	20.8^{b}	45.5 ^b
ST4	Control (no treatment)	62.3 (52.3) ^c	10.8 ^c	3.4 ^{bc}	71.8 ^c	29.1 (32.5) ^d	34.5 (35.6) ^d	52.9 ^c	17.7 ^c	39.4 ^c

Figures in parentheses are transformed angular values. The values within a column with different letters are significantly different at 5% level by using Fisher's least significance difference test.

Table 3 Effect of different foliar sprays on germination, plant height, number of hills and tillers, plant dry weight, grain yield, relative lesion size and disease index in rice during 2013

Treatment	Details	Germination	Hills	Tillers	Plant	Relative	Disease	Grains	Dry	Grain
		(%)	(no./	(no./	height	lesion height	index	(no./	weight	yield (g/
			pot)	hill)	(cm)	(%)	(%)	ear)	(g/plant)	pot)
F1	Pusa 5SD	91.7 (74.2) ^a	16.5a	10.2ab	64.3bc	19.4 (26.0) ^b	22.2 (27.8) ^c	58.2a	10.9 ^c	31.3 ^b
	(T. viride IBSD-T20)									
F2	Pusa 5SD	92.9 (75.5) ^a	16.9a	11.0a	67.0a	17.4 (24.5) ^a	18.7 (25.3) ^a	59.7 ^a	12.6a	33.3^{a}
	(T. virens IARI-P3)									
F3	Bacillus subtilis	92.5 (75.7) ^a	16.8a	10.5ab	65.7 ^{ab}	18.3 (25.2)ab	20.4 (26.6) ^b	59.0a	11.6 ^b	32.3 ^b
	(IBSD)									
F4	Hexaconazole (contaf)	90.4 (72.4) ^{ab}	16.1a	9.8 ^b	63.2 ^c	23.2 (28.4) ^c	28.7 (31.8) ^d	55.1 ^b	9.8 ^d	28.8 ^c
F5	Control (water spray)	86.7 (68.9) ^b	15.3 ^b	7.5 ^c	57.4 ^d	34.5 (35.8) ^d	45.2 (42.2) ^e	45.6 ^c	8.1 ^d	23.8^{d}

Figures in parentheses are transformed angular values. The values within a column with different letters are significantly different at 5% level by using Fisher's least significance difference test.

the disease. During 2013, plant height, number of grains/ ear grain yield, relative lesion height and disease index recorded in this interaction was statistically similar with the interaction of seed treatment with T. virens and spray with B. subtilis (ST2×F3). The interaction of seed treatment with T. virens and foliar spray of T. viride (ST2×F1) ranked third in the performance of all the variables but relative lesion height and dry plant weight were statistically similar to ST2×F3 whereas, number of grains per ear statistically did not differ with ST2×F2 during 2013. Whereas in 2014, the number of hills, tillers and grains, plant height and dry plant weight, grain yield and relative lesion height recorded in the combinations of seed treatment and foliar spray of Pusa 5SD (T. virens) and seed treatment with T. virens and foliar spray with B. subtilis did not differ significantly whereas disease index differed significantly.

Rice sheath blight caused by *R. solani* is one of the most serious diseases of rice worldwide, causing considerable yield losses (Ghosh *et al.* 2018). Since, resistant varieties are not available for the cultivation, management of the disease always a major concern. A single mode of application of chemicals/bioagents is not so effective to manage the disease as it is primary soil and seed borne, and secondary spread takes place through sclerotia and basidiospores amongst the plant populations. The present study was focused to

develop an effective management module consisting of two species of *Trichoderma*, i.e. *T. virens* and *T. viride*, bacterial antagonist *Bacillus subtilis* and fungicides hexaconazole, carbendazim and thiram in different methods of application as seed treatment and foliar spray to take care of seed borne infection as well as secondary spread of the disease.

In the present study, the foliar spray of *T. virens* followed by *B. subtilis* proved to be effective in minimizing the disease development and enhancing the yield attributing variables and grain yield. Spray of *T. viride* and hexaconazole also showed superiority over control in all the respect. The interaction of seed treatment and foliar spray of *T. virens* and seed treatment with *T. virens* and foliar spray with *B. subtilis* showed superiority in managing the sheath blight and enhancing the grain yield. Similar to the present finding Tewari and Singh (2005) also observed that a combined application of soil treatment, root dipping and foliar spray of *T. harzianum* was found most effective under green house condition.

The present study is supported by the observation of Ali and Nadarajah (2014) that the combined treatment of *Trichoderma* and *B. subtilis* suppress the *R. solani* infection. The bioagents used in the present study showed superiority over the most commonly used fungicides carbendazim + thiram for seed treatment and hexaconazole for foliar

Table 4 Effect of different foliar sprays on germination, plant height, number of hills and tillers, plant dry weight, grain yield, relative lesion size and disease index in rice during 2014

Treatment	Details	Germination (%)	Hills (no./ pot)	Tillers (no./ hill)	Plant height (cm)	Relative lesion height (%)	Disease index (%)	Grains (no./ ear)	Dry weight (g/plant)	Grain yield (g/pot)
F1	Pusa 5SD (<i>T. viride</i> IBSD-T20)	86.7 (70.0) ^a	13.3 ^{ab}	3.9 ^{ab}	75.1 ^{ab}	19.3 (25.8) ^b	19.8 (26.3) ^c	57.2ª	24.6 ^b	48.0 ^{ab}
F2	Pusa 5SD (T. virens IARI-P3)	72.5 (58.5) ^b	14.2 ^a	4.2ª	76.3 ^a	16.9 (24.1) ^a	18.0 (24.9) ^a	58.7ª	28.0a	52.3 ^a
F3	Bacillus subtilis (IBSD)	67.1 (55.0) ^c	13.8 ^{ab}	4.0^{ab}	75.3 ^{ab}	18.1 (25.0) ^b	18.5 (25.3) ^b	58.5a	25.3ab	49.6^{ab}
F4	Hexaconazole (contaf)	67.1 (55.2) ^c	12.5 ^b	3.8^{abc}	73.9 ^{bc}	21.2 (27.1) ^c	24.0 (28.9) ^d	55.9a	21.5°	45.8 ^b
F5	Control (water spray)	50.4 (45.2) ^d	10.3 ^c	3.4 ^c	71.6 ^c	31.8 (34.2) ^d	38.5 (38.2)e	52.4 ^b	17.4 ^d	35.7 ^c

Figures in parentheses are transformed angular values. The values within a column with different letters are significantly different at 5% level by using Fisher's least significance difference test.

Table 5 Effect of interaction of different seed treatment and foliar spray on germination, plant height, number of hills and tillers, plant dry weight, grain yield, relative lesion size and disease index in rice during 2013

Treatment	Details	Germination (%)	Hills (no./pot)	Tillers (no./hill)	Plant height (cm)	Relative lesion height (%)	Disease index (%)	Grains (no./ear)	Dry weight (g/plant)	Grain yield (g/ pot)
$ST1 \times F1$	Seed treatment (T. viride) × spray (T. viride)	93.3 (75.3)abc	16.3cde	11.3bcd	90.99	17.0 (24.3) ^{cde}	17.8 (24.9) ^{cd}	59.3bcd	12.0 ^{cd}	31.4 ^{de}
$ST1\times F2$	Seed treatment (T . viride) \times spray (T . virens)	93.3 (75.3) ^{abc}	16.7 ^{cd}	11.5 ^{bc}	66.5 ^b	15.3 (23.0) ^{bcd}	16.3 (23.8) ^c	59.7bcd	12.6°	34.6°
$ST1\times F3$	Seed treatment (T. viride) × spray (B. subtilis)	93.3 (77.7) ^{ab}	16.7 ^{cd}	11.5 ^{bc}	66.4 ^b	15.9 (23.5) ^{cd}	17.0 (24.4) ^{cd}	59.7bcd	12.5°	32.4 ^{cd}
$ST1\times F4$	Seed treatment (T : $viride$) × spray (hexaconazole)	93.3 (75.3) ^{abc}	16.3 ^{cde}	11cd	65.4 ^b	17.5 (24.7) ^{de}	18.5 (25.5) ^{de}	58.9cd	9.9de	30.5 ^{de}
$ST1\times F5$	Seed treatment (T : $viride$) × spray (water)	88.3 (70.1) ^{bcd}	15.3ef	7.3 ^h	60.7ef	37.5 (37.8) ^j	47.4 (43.5) ^j	47.1hi	8.0efg	23.2 ^h
$ST2 \times F1$	Seed treatment (T . virens) \times spray (T . viride)	$95.0 (79.6)^a$	17.3ab	11.7 ^b	66.5 ^b	14.8 (22.6) ^{bc}	14.1 (22.0) ^b	63.2 ^{ab}	13.1 ^{bc}	39.8b
$\text{ST2} \times \text{F2}$	Seed treatment (T . virens) \times spray (T . virens)	$96.7 (81.4)^a$	18.3a	12.3^{a}	74.2ª	$11.7 (20.0)^a$	$11.1 (19.5)^a$	64.7 ^a	18.8a	41.1a
$\text{ST2} \times \text{F3}$	Seed treatment (T . virens) \times spray (B . subtilis)	$95.0 (79.6)^a$	18ab	12.1 ^a	71.1 ^a	13.2 (21.3)ab	12.6 (20.8) ^a	64.7 ^a	14.9b	41.0^{a}
$ST2 \times F4$	Seed treatment (T : virens) \times spray (hexaconazole)	93.3 (75.3) ^{abc}	17 _{bcd}	11.6 ^b	66.5 ^b	15.3 (23.0) ^{bcd}	16.3 (23.8) ^c	60.9apc	12.7bc	34.7°
$ST2\times F5$	Seed treatment (T : virens) \times spray (water)	88.3 (70.5) ^{bcd}	$15.3^{\rm ef}$	7.98	90.5ef	30.5 (33.5) ⁱ	37.8 (37.9) ⁱ	48.5gh	8.3efg	25.68
$ST3 \times F1$	Seed treatment (carbendazim +thiram) \times spray (T . $viride$)	90.0 (72.0) ^{bcd}	16.3 ^{cde}	9.7ef	63.4bcde	20.3 (26.7) ^f	24.4 (29.6) ^f	56.9de	9.6e	27.8 ^{fg}
$\rm ST3 \times F2$	Seed treatment (carbendazim +thiram) \times spray (T : virens)	91.7 (73.4)abcd	16.3cde	10.8 ^d	65.2 _{bc}	19.1 (25.9)ef	19.3 (26.0) ^e	58.9cd	9.8de	$30.3 \mathrm{de}$
$ST3 \times F3$	Seed treatment (carbendazim +thiram) \times spray (B. subtilis)	91.7 (73.4)abcd	16.3cde	$10.2^{\rm e}$	63.7bcd	19.9 (26.5) ^f	23.7 (29.1) ^f	57.1cde	9.7e	29.4ef
$ST3 \times F4$	Seed treatment (carbendazim +thiram) \times spray (hexaconazole)	90.0 (71.6) ^{bcd}	16.3 ^{cde}	9.7ef	62.3 ^{cde}	20.3 (26.7) ^f	25.2 (30.1) ^f	55.9def	9.4ef	27.4fg
$\rm ST3 \times F5$	Seed treatment (carbendazim +thiram) \times spray (water)	88.3 (70.1) ^{bcd}	16 ^{de}	88	61.0def	25.8 (30.5) ^h	33.3 (35.2) ^h	$50.3 \mathrm{gh}$	8.9efg	25.88
$ST4\times F1$	Control (no seed treatment) × spray (T. viride)	88.3 (70.1) ^{bcd}	16 ^{de}	8.18	61.2 ^{def}	25.6 (30.4) ^h	32.6 (34.8) ^h	52.1^{fg}	6.0efg	26.0g
$\rm ST4 \times F2$	Control (no seed treatment) × spray (T. virens)	90.0 (72.0) ^{bcd}	16.3 ^{cde}	9.3 ^f	62.2de	23.7 (29.1) ^g	28.2 (32.0)g	55.3ef	9.2efg	27.3fg
$ST4 \times F3$	Control (no seed treatment) × spray (B. subtilis)	90.0 (72.0) ^{bcd}	16^{de}	8.38	61.3 ^{def}	24.1 (29.4) ^g	28.2 (32.0) ^g	54.7ef	9.2efg	26.38
$\rm ST4 \times F4$	Control (no seed treatment) × spray (hexaconazole)	85.0 (67.4) ^{cd}	$14.7^{\rm f}$	6.9h	58.4^{f}	39.7 (39.0) ^k	54.8 (47.7) ^k	44.5 ⁱ	7.2 ^{fg}	22.6h
$ST4\times F5$	Control (no seed treatment) × spray (water)	81.7 (64.7) ^d	14.3 ^f	6.8 ^h	47.38	$44.2 (41.6)^{1}$	$62.2 (52.1)^{1}$	36.7 ^j	7.18	20.5 ^h

Figures in parentheses are transformed angular values. The values within a column with different letters are significantly different at 5% level by using Fisher's least significance difference

Table 6 Effect of interaction of different seed treatment and foliar spray on germination, plant height, number of hills and tillers, plant dry weight, grain yield, relative lesion size and disease index in rice during 2014

Treatment	Details	Germination (%)	Hills (no./ pot)	Tillers (no./hill)	Plant height (cm)	Plant height Relative lesion (cm) height (%)	Disease index (%)	Grains (no./ear)	Dry weight (g/plant)	Grain yield (g/ pot)
$ST1 \times F1$	Seed treatment (T. viride) × spray (T. viride)	95.0 (79.6) ^a	13.3 ^{de}	4.0bc	75.5bcd	17.3 (24.5) ^e	17.8 (25.0) ^d	57.7abcde	23.1cd	49.7abcde
$ST1\times F2$	Seed treatment (T. viride) × spray (T. virens)	76.7 (61.3) ^{cde}	13.7 ^{de}	4.1bc	76.2abc	14.4 (22.3) ^{cd}	$16.1 (23.6)^{c}$	59.0apcd	26.7°	51.4abcd
$ST1\times F3$	Seed treatment (T. viride) × spray (B. subtilis)	68.3 (55.8)efg	13.3 ^{de}	4.1bc	76.0abcd	15.9 (23.5) ^{de}	$16.1 (23.6)^{c}$	58.8apcd	23.1cd	51.3abcd
$ST1 \times F4$	Seed treatment (T. viride) × spray (hexaconazole)	65.0 (53.8)fgh	13.0^{e}	4.0bc	75.4bcd	17.3 (24.5) ^e	18.6 (25.5) ^d	57.6abcde	22.2 ^{cde}	48.8bcde
$ST1\times F5$	Seed treatment (T. viride) × spray (water)	56.7 (48.9) ^{hij}	10.3^{fg}	3.4°	71.6 ^{def}	30.6 (33.6) ^{jk}	36.0 (36.9) ^j	52.7def	17.5ef	36.4 ^{def}
$ST2 \times F1$	Seed treatment (T. virens) × spray (T. viride)	90.0 (72) ^b	15.7bc	4.4abc	77.6ab	13.7 (21.4) ^{abc}	13.9 (21.9) ^b	61.7 ^{ab}	35.5 ^b	52.8abc
${\rm ST2}\times{\rm F2}$	Seed treatment $(T. virens) \times spray (T. virens)$	73.3 (59) ^{def}	17.7 ^a	5.4^{a}	80.2^{a}	$11.7 (19.9)^a$	$11.9 (20.1)^a$	64.7^{a}	43.9 ^a	64.0^{a}
$\mathrm{ST2}\times\mathrm{F3}$	Seed treatment (T. virens) × spray (B. subtilis)	68.3 (55.8)efg	17.0ab	4.8^{ab}	77.7ab	12.4 (20.6) ^{ab}	12.8 (20.9) ^{ab}	64.5 ^a	38.3 ^b	56.6 ^{ab}
$ST2\times F4$	Seed treatment (T virens) \times spray (hexaconazole)	80.0 (63.5) ^{cd}	15.0^{cd}	4.2abc	76.3abc	14.2 (22.1) ^{bcd}	$15.6 (23.3)^{\circ}$	60.0^{abc}	26.7°	52.5abc
$ST2\times F5$	Seed treatment $(T. virens) \times \text{spray (water)}$	$55.0 (48)^{ij}$	10.7^{fg}	3.4°	72.5cdef	28.9 (32.5) ^j	$32.7 (35.1)^{i}$	53.2cdef	18.0 ^{def}	37.8cdef
$ST3 \times F1$	Seed treatment (carbendazim +thiram) \times spray (T : $viride$)	85.0 (67.4) ^{bc}	12.3°	3.7bc	74.5bcde	20.7 (27.0) ^{fg}	21.0 (27.3) ^e	55.2bcdef	21.5cde	46.9bcde
$ST3 \times F2$	Seed treatment (carbendazim +thiram) \times spray (T : $virens$)	70.0 (56.8) ^{efg}	13.0°	3.9bc	74.8 ^{bcd}	19.5 (26.2) ^f	20.6 (26.9) ^e	56.7bcdef	21.8cde	48.4bcde
$\text{ST3} \times \text{F3}$	Seed treatment (carbendazim +thiram) \times spray (B. subtilis)	66.7 (54.7) ^{fgh}	12.7e	3.7bc	74.5bcde	20.2 (26.7) ^{fg}	20.9 (27.0) ^e	56.5bcdef	21.5cde	47.0bcde
$ST3 \times F4$	Seed treatment (carbendazim +thiram) × spray (hexaconazole)	61.7 (51.8)ghij	12.3°	3.6bc	73.9bcdef	21.1 (27.4) ^{fg}	21.8 (27.2) ^e	54.6bcdef	20.9def	46.2 ^{bcde}
$ST3 \times F5$	Seed treatment (carbendazim +thiram) \times spray (water)	51.7 (46) ^j	12.7e	3.4°	72.7cdef	26.0 (30.7) ⁱ	27.3 (27.8) ^{ef}	53.4cdef	18.2 ^{def}	38.8cdef
$ST4\times F1$	Control (no seed treatment) \times spray (T . viride)	76.7 (61.2) ^{cde}	$12.0^{\rm ef}$	3.5°	72.8cdef	$25.6 (30.4)^{i}$	26.5 (31.5) ^h	54.3cdef	18.5 ^{def}	42.6bcdef
$ST4 \times F2$	Control (no seed treatment) \times spray (T . virens)	70.0 (56.8)efg	12.3e	3.5°	73.5bcdef	22.2 (28.1)gh	$23.5 (29.0)^{fg}$	54.3cdef	19.5def	45.6bcde
$ST4\times F3$	Control (no seed treatment) \times spray (B. subtilis)	65.0 (53.7) ^{fghi}	12.3e	3.5°	72.8cdef	23.9 (29.3)hi	24.2 (29.5) ^g	54.3cdef	18.4^{def}	43.7bcdef
$ST4 \times F4$	Control (no seed treatment) \times spray (hexaconazole)	61.7 (51.8)ghij	9.78	3.30	$70.2^{\rm ef}$	32.3 (34.6) ^k	$40.0(39.2)^{k}$	$51.5^{\rm ef}$	16.1^{f}	35.5ef
$ST4\times F5$	Control (no seed treatment) × spray (water)	38.3 (38.2) ^k	7.7h	3.2°	₁ 9.69	$41.7 (40.3)^{1}$	$58.0 (49.6)^{1}$	$50.3^{\rm f}$	16.0^{f}	29.7 ^f
Figures in	Fioures in parentheses are transformed anoular values. The values within a column with different letters are sionificantly different at 5% level by using Fisher's least sionificance difference	within a column	with differen	nt letters are	- sionificantly	different at 5%	level hy using F	isher's leas	t sionificanc	- difference

Figures in parentheses are transformed angular values. The values within a column with different letters are significantly different at 5% level by using Fisher's least significance difference

spray. This is also supported by the observation made by Naeimi *et al.* (2010) that *T. harzianum* strain AS 12-2 proved better than the most commonly used fungicide propiconazole in Iran against sheath blight of rice. Mondal (2012) also reported that *Trichoderma* isolate B-18 and B-16 increased the yield and reduced the sheath blight incidence significantly and recommended to be used as bio-fungicide as well as bio-fertilizer for the management of sheath blight of rice.

The results of present study clearly indicate that *T. virens* based Pusa 5SD as seed treatment and foliar spray either with *T. virens/T. viride* or *B. subtilis* could be used to manage sheath blight and increase grain yield of rice.

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REFERENCES

- Ali H and Nadarajah K. 2014. Evaluating the efficacy of *Trichoderma* spp and *Bacillus subtilis* as biocontrol agents against *Magnaporthe grisea* in rice. *Australian Journal of Crop Sciences* 8: 1324–35.
- Boukaew S, Klinmanee C and Prasertsan P. 2013. Potential for the integration of biological and chemical control of sheath blight disease caused by *Rhizoctonia solani* on rice. *World Journal of Microbiology and Biotechnology* **29**: 1885–93.
- Chen L, Zhanga J, Shaoa X, Wangb S, Miaoa Q, Maoa X, Zhaia Y and Shea D. 2015. Development and evaluation of *Trichoderma asperellum* preparation for control of sheath blight of rice (*Oryza sativa* L.). *Biocontrol Science and Technology* **25**: 316–28.
- Da Silva J C, Torres D B, Lustosa D C, De Filippi M C C and Da Silva G B. 2012. Rice sheath blight biocontrol and growth promotion by *Trichoderma* isolates from the Amazon. *Amazonian Journal of Agricultural and Environmental Sciences* 55: 243–50.
- Das B C and Hazarika D K. 2000. Biological management of sheath blight of rice. *Indian Phytopathology* **53**: 433–5.
- Dath P.A. 1990. *Sheath Blight Disease of Rice and its Management*. Associated Publishing Company, New Delhi, p 129.
- Dubey S C and Patel B. 2001. Evaluation of fungal antagonists against *Thanatephorus cucumeris* causing web blight of urd and mung bean. *Indian Phytopathology* **54**: 206–9.
- Dubey S C, Tripathi Aradhika and Singh Birendra. 2012. Combination of soil application and seed treatment formulations of *Trichoderma* species for integrated management of wet root rot caused by *Rhizoctonia solani* in chickpea. *Indian Journal of Agricultural Sciences* 82: 357–64.
- Dubey S C, Bhavani R and Singh B. 2009. Development of Pusa 5SD for seed dressing and Pusa Biopellet 10G for soil application formulations of *Trichoderma harzianum* and their evaluation for integrated management of dry root rot of mungbean (*Vigna radiata*). *Biological Control* 50: 231–42.
- Dubey S C, Bhavani R and Singh B. 2011. Integration of soil application and seed treatment formulations of *Trichoderma* species for management of wet root rot of mungbean caused by *Rhizoctonia solani*. *Pest Management Science* **67**: 1163–8.
- Ghosh S, Kanwar P and Jha G. 2018. Identification of candidate pathogenicity determinants of *Rhizoctonia solani* AG1-IA, which causes sheath blight disease in rice. *Current Genetics* **64**: 729–40.

- Gomez K A and Gomez A A. 1984. *Statistical Procedures for Agricultural Research*, pp 39–153. John Wiley & Sons, Singapore.
- Groth D E and Bond J A. 2006. Initiation of rice sheath blight epidemics and effect of application timing of Azoxystrobin on disease incidence, severity, yield and milling quality. *Plant Disease* **90**: 1073–8.
- Groth D E, Rush M C and Hollier C A. 1991. *Rice Diseases and Disorders in Louisiana*. Louisiana State University, Agricultural Center, Louisiana Agricultural Experiment Station, Bulletin No. 828. Baton Rouge, LA, USA.
- Guo T and Liao M. 2014. Suppression of *Rhizoctonia solani* and induction of host plant resistance by *Paenibacillus kribbensis* PS04 towards controlling of rice sheath blight. *Biocontrol Science and Technology* **24**: 116–21.
- Harman G E. 2006. Overview of mechanisms and uses of *Trichoderma* spp. *Phytopathology* **96**: 190–4.
- Harman G E, Howell C R, Viterbo A, Chet I and Lorito M. 2004. Trichoderma species opportunistic, avirulent plant symbionts. Nature Review of Microbiology 2: 43–56.
- IRRI. 2002. Standard Evaluation System for Rice. International Rice Research Institute, Philippines.
- Mondal G. 2012. Plant growth promoting activity of some indigenous *Trichoderma* isolates and their field performance against sheath blight of rice in old alluvial zone of North Bengal. *The Journal of Plant Protection Sciences* 4: 16–24.
- Naeimi S, Okhovvat S M, Javan-nikkhah M, Vagvolgyi C, Khosravi V and Kredics L. 2010. Biological control of *Rhizoctonia solani* AG1-1A, the causal agent of rice sheath blight with *Trichoderma* strains. *Phytopathologia Mediterranea* **49**: 287–300.
- Oard S, Rush M C and Oard J H. 2004. Characterization of antimicrobial peptides against a US strain of the rice pathogen *Rhizoctonia solani*. *Journal of Applied Microbiology* **97**: 169–80.
- Schisler D A, Slininger P J, Behle R W and Jackson M A. 2004. Formulation of *Bacillus* spp. for biological control of plant diseases. *Phytopathology* **94**: 1267–71.
- Shekhada M R, Patil V A, Savaliya A S and Sangani M D. 2018. Management of rice (*Oryza sativa* L.), sheath blight (*Rhizoctonia solani* Kuhn) and sheath rot (*Sarocladium oryzae* Sawada) through seed bio-priming. *International Journal of Current Microbiology and Applied Science* 7: 2787–94.
- Soe K T and De Costa D M. 2012. Development of a spore-based formulation of microbial pesticides for control of rice sheath blight. *Biocontrol Science and Technology* **22**: 633–57.
- Srinivasachary S, Willocquet L and Savary S. 2011. Resistance to rice sheath blight (*Rhizoctonia solani* Kühn) [(teleomorph: *Thanatephorus cucumeris* (A.B. Frank) Donk.] disease: Current status and perspectives. *Euphytica* **178**: 1–22.
- Tewari L and Singh R. 2005. Biological control of sheath blight of rice by *Trichoderma harzianum* using different delivery systems. *Indian Phytopathology* **58**: 35–40.
- Wiwattanapatapee R, Chumthong A, Pengnoo A and Kanjanamaneesathian M. 2013. Preparation and evaluation of *Bacillus megaterium* -alginate microcapsules for control of rice sheath blight disease. *World Journal of Microbiology and Biotechnology* 29: 1487–97.
- Yang X, Chen L, Yong X and Shen Q. 2011. Formulations can affect rhizosphere colonization and biocontrol efficiency of *Trichoderma harzianum* SQR-T037 against *Fusarium* wilt of cucumbers. *Biology and Fertility of Soils* 47: 239–48.