

Original Research Article

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Biocontrol of *Sclerotium oryzae* by *Pseudomonas fluorescens* and *Trichoderma* spp. Isolated from Rice Rhizosphere of Indo- Burma Biodiversity Hotspot with Reference to Manipur

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ABSTRACT

Stem rot of rice (*Oryza sativae* L.) caused by *Sclerotium oryzae* Catt. is found to occur frequently in Manipur infecting all cultivated lowland rice cultivars and become a major concern in rice production. Local strains of *Pseudomonas fluorescens* and *Trichoderma* collected from rice fields of Imphal East and Imphal West districts of Manipur were screened for their ability to control *S. oryzae* and production of different biocontrol mechanisms under laboratory conditions. Further, combined application of talc formulations of selected strains of *P. fluorescens* IE 62 and *T. inhamatum* (T 80) (based on *in vitro* antifungal activity, production of cell wall degrading enzymes and secondary metabolites) were assessed for their plant growth promotion and biocontrol ability under greenhouse and field conditions. Seed germination, root length, shoot length and plant height were enhanced by treatment with *P. fluorescens* IE 62 and *T. inhamatum* T 80 *in vitro* conditions as compared to single applications under greenhouse and field conditions. Field data revealed significant reduction in stem rot incidence, lesion number and size when applied in consortia. Percent reduction in lesion number and size were recorded as 35.15% & 26.14% when applied with *P. fluorescens* IE 62 alone and 51.63% & 14.43% with combined applications as compared to control plot indicating better biocontrol activity. The results indicated the effectiveness of combined application of local strains of *P. fluorescens* IE 62 and *T. inhamatum* T 80 resulting increased plant growth and control of *S. oryzae* and therefore can be used as potential bioagents for managing Stem rot disease in rice.

Keywords

Pseudomonas fluorescens,
Trichoderma spp.,
Stem rot, Rice,
Biocontrol,
Sclerotium oryzae

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Introduction

Stem rot of rice caused by *Sclerotium oryzae* is present in all rice growing regions

worldwide (Ou, 1985). Its potential threat to reduction in rice yield has become a major concern in rice cultivation and production (Kumar *et al.*, 2003). Yield losses ranges

from 30-80% in Phillippines (Ou 1985) and 10-70% in India (Singh and Pavgi 1966). In Manipur, it has infected all cultivated lowland rice cultivars either of local or exotic origin to Manipur (Konhoujam *et al.*, 2007). Disease incidence of 40% was recorded at maturity stage. The exclusive and constant monoculture of rice with no alternate crop rotation practices coupled with the unavailability of disease resistant rice cultivars has aggravated the severity of stem rot of rice in the state which becomes a threat to production and yield of rice. The disease is now endemic in Manipur (Konhoujam 1998).

The use of beneficial micro-organisms as biocontrol agents has become more important in recent years not only to improve plant growth and to manage plant diseases but also to avoid environmental pollution. Being eco-friendly and cost effective strategy, it can be used in integration with other strategies for a greater level of protection with sustained rice yields. Among the biocontrol agents, *Pseudomonas fluorescens* and *Trichoderma* are most commonly used against rice diseases (Vasudevan *et al.*, 2002). *Pseudomonas* spp. because of their ability to colonize the rhizosphere of host plants (widely prevalent in rice rhizosphere) and ability to produce a wide range of compounds inhibitory to a number of plant pathogens (Anjaiah *et al.*, 1998; Weller 1988; Copper and Higgins 1993; Vidhyasekharan and Muthamilan 1995). *Trichoderma* spp. is one of the most potential fungal biocontrol agent used against soil/ seed borne fungal diseases of several crop plants (Kubicek *et al.*, 2001). Combination of seed treatment, soil application and foliar spray with *P. fluorescens* recorded the minimum disease incidence of bacterial leaf blight with maximum yield (Jeyalakshmi *et al.*, 2010). Increased root and shoot lengths, dry weight and plant height were recorded following treatment of plants with *P. fluorescens* and *T.*

viride either alone or in combination when compared with control. Application of *P. fluorescens* and *T. viride* resulted in a significant reduction of sheath blight incidence (Mathivanan *et al.*, 2005). *Pseudomonas fluorescens* inhibited the growth of the sheath blight pathogen, *R. solani* by the production of antibiotics (Gaffney *et al.*, 1994) and siderophores (Savitry and Gnanamanickam, 1987) whereas *T. viride* degraded the chitin polymers from the cell wall of *R. solani* by secreting chitinase (Krishnamurthy *et al.*, 1999). The use of multiorganisms as crop production and crop protection inputs is currently under practice in agriculture. Further combination of biocontrol agents was reported to offer an effective control of plant diseases (Duijff *et al.*, 1999; De Boer *et al.*, 2003). Combined inoculation of *P. fluorescens* with symbiotic nitrogen-fixing bacteria has been reported to promote plant growth and reduce the disease incidence (Nishijima *et al.*, 1988). Increased root and shoot lengths, dry weight and plant height were recorded following treatment of plants with *P. fluorescens* and *T. viride* either alone or in combination with significant reduction of sheath blight incidence (Mathivanan *et al.*, 2005). Combined use of biocontrol agents with different mechanisms is reported to improve disease control and also to overcome the inconsistent performance of the introduced biocontrol agents and could be more effective in controlling soil borne pathogens than a single agent (Nelson 2004). Strains of *P. fluorescens* and *Trichoderma* spp. are potential biocontrol agents for controlling foot rot disease in black pepper (Sharma *et al.*, 2000), stem rot in groundnut (Manjula *et al.*, 2004), wilt of tomato (Rini and Sulochana, 2007), etc. In North East India, such efforts have been tried by few with unsatisfactory output. Therefore, in this study, an attempt has been made to check the combined effect of local strains of *P. fluorescens* and *Trichoderma* spp. on crop

growth and control of Stem rot of rice besides screening various biocontrol mechanisms.

Materials and Methods

Isolation and identification of causal organism of Stem rot of rice from infected rice fields

Stem rot infected leaf samples were collected from various locations of rice fields of Manipur. To isolate the pathogen, surface sterilized small bits of infected leaves were inoculated in PDA medium under aseptic condition and incubated at 28±°C for 7-10 days. Pathogen was then identified based on colony characteristics and morphological structures as well as molecular identification based on ITS amplification and was compared with the reference strain, ITCC 4107 obtained from IARI, New Delhi. The causal organism was found to be *Sclerotium oryzae* as confirmed by Koch's Postulates experiment.

Isolation of *P. fluorescens* strain from rice rhizosphere of 5 different locations of Manipur

Soil samples from rice rhizosphere of five different locations namely Kongba Uchekon, Kongpal and Yaralpat of Imphal East and Phayeng and Takyelpat of Imphal West were collected to isolate the bacteria using serial dilution method in King's B medium. Single colonies showing characteristic fluorescens colour when exposed to UV at 365 nm were selected and sub cultured on LB broth which were then cryopreserved at -80°C in 20% glycerol for further studies.

In vitro* antagonistic activity of local *P. fluorescens* and *Trichoderma spp.* against *S. oryzae

A total of 158 *P. fluorescens* strains isolated from rice rhizosphere were screened for their

antagonistic action against the fungus, *S. oryzae* by dual culture assay. The bacteria were streaked at the periphery of PDA plates (pH 6.1) with 9 cm diameter. After 24 h of incubation at 30° C, a 6 mm disc of an actively growing culture of *S. oryzae* was inoculated at the center. Plates inoculated with *S. oryzae* alone were maintained as control. All the inoculated plates were further incubated for 72 h at 28°C and the colony diameter in each treatment was compared with that of control. The percentage inhibition was calculated with the help of the formula given by Whipps (1997).

A total of 5 IBSD *Trichoderma* isolates with proven biocontrol potential (Kamala and Indira, 2011) collected from different ecological niches of Manipur were screened for their antagonistic potential against *S. oryzae*. Mycelial discs of 6 mm diameter from actively growing cultures of *Trichoderma spp.* were inoculated at either end of PDA and incubated for 7 days at 28°C. The plates were observed at regular intervals of 24 h and the antifungal activity was recorded on a 1-5 rating scale (Bell *et al.*, 1982). PDA plates inoculated with *S. oryzae* alone were treated as control. The above experiments were repeated with three replications.

Compatibility test of *Trichoderma spp.* and *P. fluorescens* isolate

Trichoderma isolate T 80 that showed maximum inhibition of *S. oryzae in vitro* was selected for checking compatibility with *P. fluorescens* isolate IE 62. For this, a small portion from the single colony of IE 62 was inoculated on one edges of the PDA plate. After one day, 6 mm disc of 7 days old mycelium of *Trichoderma* were taken and inoculated on the opposite side of the inoculated *P. fluorescens* isolate and incubated for seven days. *Trichoderma spp.* which can grow independently with the *P.*

fluorescens isolate IE 62 on PDA plate was selected for effectivity trial under greenhouse and field conditions.

Screening of different biocontrol mechanisms exhibited by *P. fluorescens* isolates

Five different *P. fluorescens* strain and five IBSD *Trichoderma* isolates that showed maximum antagonistic activity against *S.oryzae* were screened for various biocontrol mechanisms which are given below:

Protease and chitinase

Protease activity of local strains of *P. fluorescens* and *Trichoderma* were determined from clearing zones in skim milk agar after five – seven days of incubation at 28°C (Berg *et al.*, 2002).

Chitinase activity was tested on chitin minimal medium according to the method of Chernin *et al.*, 1995 for bacterial isolates. Clearing zones indicating the enzymatic degradation were measured after 1-7 days of incubation. For *Trichoderma* isolates, it was determined on chitin detection medium (Roberts and Selitrennikoff, 1988). Purple zone formation around the mycellia indicates chitinase production.

Siderophore and Hydrogen cyanide (HCN) production

Siderophore was assayed by plate method using Ternary complex chrome azurol S (CAS), Fe³⁺/ Hexadecyl trimethyl ammonium bromide (HDTMA) as an indicator (Schwyn and Neilands 1987). Formation of a bright zone with a yellowish fluorescens in the dark blue medium indicates production of siderophore by the bacterial isolates. The HCN production by *P. fluorescens* was determined using picric acid solution by

observing the colour change from yellow to brown and reddish brown. Scoring was done as weak- yellow to light red, moderate-brown, strong- reddish brown (Millar and Higgins 1970).

Phosphorus Solubilization (PS)

Bacterial cultures were streaked on Pikovskayas agar (PKA) to check phosphorus solubilizing ability. Those isolate forming clear zones were scored positive (Pikovskaya 1948)

Sequence analysis

The identity of the bacterial isolate IE 62 was confirmed by 16S rDNA sequences and BIOLOG based identification and showed it to be 97% similar to that of *Pseudomonas fluorescens* (EU5544291). ITS amplification of the isolated fungus from stem rot infected leaf samples showed that the sequence was similar to that of *Ceratorhiza oryzae – sativae* (FJ6672571) showing 100% similarity which is a synonym of *Sclerotium oryzae sativae*. ITS amplification of the isolated *Trichoderma* isolate T 80 showed that the sequence was similar to that of *Trichoderma inhamatum* (GQ426033) showing 97% similarity.

Plant growth and disease control ability trial in phytochamber, greenhouse and field

Rice var. KD was used as a test crop for all the experiments. The bacterial and fungal formulation with presterilized talc powder was prepared as described by Vidyasekaran *et al.*, (1997). The population of *P. fluorescens* IE62 in the talc formulation was 13.3×10^{10} cfu/g and for *T.inhamatum* T80 it was 1×10^6 conidia/ ml) at the time of use. For observing seed germination, root and shoot length, seeds were soaked for 15 minutes in formulation (20 g l^{-1}) of *P. fluorescens* IE62 and

T.inhamatum T80 and incubated in a growth chamber at 28±2° C. The types of treatment were i) *P. fluorescens* IE62, ii) *P. fluorescens* IE62 + *T.inhamatum* T80 and iii) Control. Germination rate, root and shoot length were recorded after 5-6 days.

Pot experiment was laid out in a CRD with three replications (3 plants/ pot sizes of 25x 30 cm) containing mixture of FYM, sand and soil to the ratio of 1/2:1:2 in greenhouse. The pathogen inoculum (*S.oryzae*) cultured in autoclaved rice grains were inoculated in the potting mixture. After two days, formulations were applied to the soil (15g/pot) and germinated rice seedlings of 15 days old were transferred to the potting mixture after giving root dip treatment (20g l⁻¹) for 15 mins. The treatments were i) *P. fluorescens* -IE62 (soil application and root dip treatment), ii) *S.oryzae* (S.o); (soil application), iii) *S.oryzae* (S.o) + *P. fluorescens* IE62+ *Trichoderma inhamatum* T80 (soil application + root dip treatment) and iv)Control (non-treated).

In order to confirm the result obtained in greenhouse trial, field experiment (plot sizes;15 x 20 ft) laid out in a RBD was conducted for two consecutive years, 2010 and 2011 in Stem rot prone areas of Phayeng, Imphal West District of Manipur. FYM (100 kg/ ha) was added to the plots one month ahead of transplanting the seedlings. The treatments given were as follows:

- i) *P. fluorescens* IE62 (seed treatment + soil application + root dip treatment)
- ii) *P. fluorescens* IE62 + *T.inhamatum* T80 (seed treatment + soil application + root dip treatment)
- iii) Control (seeds soaked in LB broth)

The observations were recorded on different parameters viz. plant growth, stem rot incidence and lesion formation. Stem rot incidence was calculated by applying the standard formula given by Mc Kinney, 1923.

Statistical analysis

Different treatments in all the experiments were arranged in a completely randomized block design. Values given in the tables are means based on replicates. Data from all the experiments were analyzed by analysis of variance (ANOVA) using Genstat 5 statistical package. Least significant difference (LSD) at 5% level of significance ($P=0.05$) was used to compare the mean values of different treatments in an experiment. Pooled data of two consecutive years of the greenhouse and field experiments were subjected to ANOVA.

Results and Discussion

In vitro antifungal activity of local *P. fluorescens* isolates and *Trichoderma spp.* against *S. oryzae*

Among the 158 *P. fluorescens* strain screened for antifungal activity against *S. oryzae*, five isolates showed maximum biocontrol potentials. Among them, *P. fluorescens* IE 62 showed maximum inhibition of *S. oryzae* with 40.52% (Fig. 1A) followed by IE 182 (38.26%) and IE 23 (28.56%) respectively (Table 1). A potential IBSD *Trichoderma* isolates, *T. inhamatum* T 80 showed maximum mycellial inhibition of *S. oryzae* with 80.23% (Fig. 1B). Antifungal activity of the local strains of *P. fluorescens* was compared with the reference *P. fluorescens* strain- 103 obtained from IMTECH, Chandigarh which failed to inhibit growth of *S. oryzae* even after seven days of incubation (Table 1, Fig. 1C).

Screening for various biocontrol mechanisms of *P. fluorescens* strain and *Trichoderma T80*

All the five isolates showed protease and siderophore production. IE 182 did not show chitinase activity. Hydrogen cyanide

production was shown by two isolates namely IE 17 and IE 182 whereas phosphorus solubilization was shown by isolates IE 18, IE 62 and IE 182 (Table 2). Very low protease activity was exhibited by *P. fluorescens* strain 103 with clearance zone of only 5mm which was lower as compared to all the five isolates screened. *Trichoderma* T80 showed protease activity with clearance zone diameter of 37.67 mm and chitinase activity with purple zone of 81.33mm.

***In vitro* compatibility of *P. fluorescens* IE 62 and *T. inhamatum* T 80**

Based on antifungal and biocontrol mechanisms exhibited by different *P. fluorescens* strain, isolate IE 62 was selected for conducting greenhouse and field trials. For combined effectiveness trial, compatibility test of *T. inhamatum* T 80 and *P. fluorescens* isolate IE was done and were found compatible to each other (Fig. 1D). Thus, *T. inhamatum* T 80 was selected for combined application with *P. fluorescens* isolate IE 62 for greenhouse and field trial.

Effect of single and combined application on seed germination, root and shoot length of rice seedlings var. KD in vitro conditions

Both single and combined treatment of IE 62 and T 80 significantly enhance the germination rate. Single application with IE 62 gave 90.92% seed germination and combined application (IE 62+ T 80) gave 90% seed germination enhancing it by 9.32% and 8.89% respectively as compared to control which recorded 82.45% seed germination (Table 3). Length of root and shoot of the treated seedlings were recorded one week after germination. Both single and combined application significantly increased the root and shoot length as compared to control. Percent increase in root length (50.6 mm) was 17.38 and shoot length (8.55mm) -

3.27 respectively in combined application as compared to single application which recorded 44.4 mm root length and 52 mm shoots length with increase of 0.59% and 3.27% respectively (Table 3; $P_{(0.05)} = 0.0011$ -root), 4.38- shoot).

Effect of treatment of *P. fluorescens* IE 62 and *Trichoderma* T 80 on height of rice cultivar KD in greenhouse and field trial

Height of rice plant was recorded 35 days after planting under green house conditions (Table 4). Plant height was found to be significantly increased with both single (58.76cm) and combined treatment (59.9cm), combined application being more effective with 4.84% increase in height and single treatment - 3.01% as compared to control which recorded height of 57 cm only (Table 4; $P_{(0.05)} = 1.19$). In order to confirm the result obtained in greenhouse trial, field experiment was conducted at Imphal West district of Manipur for two consecutive years i e 2010 & 2011. Data represented in table 4 is pooled data of two years. Plant height was recorded three months after planting. In this experiment, combined application(IE 62+ T 80) showed significant increase in plant height with 8.84% whereas plants treated with IE 62 alone showed only 2.48% as compared to control (Table 4; $P_{(0.05)} = 4.26$).

Effect of treatment of *Pseudomonas* and *Trichoderma* on lesion formation of *S. oryzae* under green house and field conditions

Number and size of lesions produced by *S. oryzae* on infected leaf samples were recorded 45 days after giving secondary infection by foliar spray method. Both single and combined treatment significantly reduced lesion number and lesion size as compared to control which is infected only with *S.oryzae* (Figure 3). Combined treatment (*S.o*+ IE 62+

T 80) showed significant difference recording 38.83% and 49.23% reduction in lesion number and size respectively when compared with single treatment of 22.17% and 27.69% reduction irrespective of lesion number ($P_{(0.05)} = 0.0024$) and size ($P_{(0.05)} = 0.02$).

In field trial, similar observations were obtained. Infected plant samples of 45 days old were collected from different treatments (Figure 4) and number and size of lesions were recorded. Field data indicated significant reduction in both lesion number and size by single treatment with IE 62 as well as combined treatment with *T. inhamatum* (T 80) as compared to control field (Figure 4a) which was infected only with *S. oryzae* under natural conditions. Single treatment (IE 62) recorded less lesion number (14.67) and lesion size (5.99 mm) with % reduction of 51.63 and 26.14 respectively (Figure 3, 4b) as compared to combined treatment (IE 62+ T 80) which recorded 19.67 lesion number and 6.94 mm lesion size with 35.15% and 14.43% decrease respectively (Figure 2, 3 and 4c) and the treatments were found statistically significant.

Effect of treatment of *Pseudomonas* and *Trichoderma* on stem rot incidence in greenhouse and field conditions

Incidence of Stem rot was recorded for each treatment after two months of planting in greenhouse conditions. Combined application (S.o + IE 62 + T 80) resulted in less disease incidence (43.95%) and single application (S.o + T 80) recorded 61.97% as compared to control which recorded stem rot incidence of 72 % (Table 5). This result clearly indicates that combined application of *P. fluorescens* IE 62 and *Trichoderma* T 80 gave better control of stem rot as compared to single application.

Similar observations were obtained from field trial with combined application recording stem rot incidence of 40.47% than single

treatment with *P. fluorescens* IE 62 that recorded 48.28% as compared to control which recorded 100% stem rot incidence (Table 5).

The present findings identified many potential *Pseudomonas* strains from rice rhizosphere of different locations of Manipur of which many of them were antagonistic against the newly isolated pathogen ie *S. oryzae*. A total of twenty *P. fluorescens* isolates were found to inhibit *S. oryzae* in dual culture assay with differing range of inhibition zone.

The bacterial isolates were found to exhibit multiple cell wall degrading enzymes and secondary metabolites which might have contributed in pathogenesis. In the present study, the selected antifungal *P. fluorescens* isolates IE 62 were observed to produce protease, chitinase, siderophore, HCN *in vitro*, which might have contributed for their maximum biocontrol ability in addition to antibiotics. A positive relationship was observed between the antifungal activity of chitinolytic *P. fluorescens* isolates and their level of chitinase production (Velazhahan *et al.*, 1999). In contrast to the mycelial inhibition in dual cultures, all the five *P. fluorescens* isolates differed in their biocontrol ability possibly due to the differences in root colonization and production of antifungal metabolites in natural environments. HCN and siderophores produced by *Pseudomonas* spp. were also involved in their antifungal activity. Voisard *et al.*, (1989) observed that suppression of black rot of tobacco was due to the production of HCN by *P. fluorescens* which also induced resistance in the host plant. Antagonistic assay of *T. inhamatum* T 80 against *S. oryzae* *in vitro* conditions resulted in maximum inhibition of *S. oryzae* possibly due to the parasitization and colonization of sclerotia as reported by Haroon Usmani (1980) and Henis *et al.*, (1983).

Combination of different biocontrol agents is often observed as an effective means for sustained disease control. Combined application of *P. fluorescens* IE 62 and *T. inhamatum* T 80 resulted in improved biocontrol both in terms of plant growth and disease management than single application with only *P. fluorescens* IE 62 as shown by greenhouse screening and field trials. Seed germination, root and shoot length were enhanced by combined treatment as compared to single treatment. Similar observations were obtained by Singh and Arora (2001) who showed that growth parameter and vigor

index were high in *Trichoderma* sp and *P. fluorescens* treatment. Plant growth may have improved due to growth regulators produced by the antagonists together with their continuous supply to the developing plants as a result of the intimate contact between the seeds and the biocontrol agent (Tarek and Moussa, 2002). Application of *P. fluorescens* and *T. viride* either alone or in combination showed a positive influence in improving growth attributes in rice such as shoot and root lengths and dry weight, plant height and time up to 50% flowering when compared with fungicide treatment and control.

Table.1 Antagonistic activity of 20 selected local rhizobacteria in dual plate assay

Sl. No	Bacterial isolates	Antagonistic activity	
		<i>S. oryzae</i>	% of growth inhibition*
1.	IE 1	+	12.36
2.	IE 2a	+	10.1± 0.25
3.	IE 14	+	10.42± 0.97
4.	IE 16	+	9.33± 0.85
5.	IE 17	++	25.49± 0.41
6.	IE 18	++	27.15± 1.31
7.	IE 23	++	28.56± 1.03
8.	IE 182	++	38.26± 1.09
9.	IE 271	++	17.91±0.05
10.	IE 62	+++	40.52± 0.84
11.	IE 326	++	22.91± 1.5
12.	IE 332	++	20.23±0.42
13.	IE 323	+++	36.78± 0.46
14.	IB 137	+	14.78± 0.29
15.	IB 371	+	11.15±1.12
16.	IB 477	++	18.95± 0.11
17.	IB 488	++	19.18± 0.2
18.	IB 546	++	20.61± 0.27
19.	C 37	+	14.5± 0.65
20.	C 51	+	13.05± 1.54
21	Control	-	-
LSD(P=0.05)			1.16

* Mean of three replications

Table.2 Biocontrol mechanisms of exhibited by local rhizobacterial isolates

Sl. No	Rhizobacteria	Different biocontrol mechanisms exhibited by local rhizobacterial isolates.					
		Zone diam.(mm)				HCN	PS
		Protease*	Chitinase*	Siderophore*	Glucanase		
1.	IE 1	25±0	10±1.15	18±0	++	-	-
2	IE 2a	15±0	12±0	12±0.76	+	-	-
3.	IE 14	23±0.86	12±0.86	17±0.86	+	-	-
4.	IE 16	24±0.69	10±1.73	15±0	++	-	-
5	IE 17	20±2.42	10±0.86	-	++	+	-
6	IE 18	23±1.15	-	12.0	++	-	+
7	IE 23	24±0.28	-	17±0.57	++	-	-
8	IE 271	21±0.28	-	16.0	+	+	+
9	IE 62	18±0	15.3±0.4	13.3±0.40	++	+	+
10	IE 326	16±0	10±0	15±0.57	-	+	-
11	IE 332	16±0.58	12±0.86	14±0	-	+	-
12	IE 323	15±0.17	10±1.15	15±0.58	+++	+	+
13	IB 477	22.67±1.33	10.1±0.73	15±0.09	++	-	+
14	IB 488	19.3±0.43	12.9±0.06	12±0	-	+	+
15	IB 546	14.5±0	-	14.9±0.03	++	+	-
16.	IB 137	14±1.15	7.0±0.57	-	+	+	-
17	IB 371	16±0.86	12±0.57	14±0.57	+	+	-
18.	IE 182	13±0.57	-	15.0±0.28	+	+	-
19.	C 37	18±0.57	10±0.57	12±1.44	-	+	+
20.	C 51	14±0	-	-	-	-	-
L.S.D (P=0.05)		3.82	1.0	4.94		1.0	

* Mean of three replications, ± SE - Standard Error

Table.3 Effect of treatment of *Pseudomonas* and *Trichoderma* on seed germination, root and shoot length of rice var. KD after 7 days of incubation

Sl.no	Type of seed treatment	% of seed germination	Root		Shoot	
			Length (mm) *	% increase in length(mm) *	Length (mm) *	%increase in length(mm) *
1.	IE 62	90.92± 0.47	44.3± 8.45	17.38	52.0± 2.31	3.27
2.	IE 62+ T 80	90± 0	50.6± 6.36	0.59	55.0± 0.58	8.55
3.	Control	82.45± 4.33	36.6± 1.67	-	50.3± 2.33	
LSD(P= 0.05)		2.18	4.16		6.48	

Table.4 Effect of treatment of *Pseudomonas* and *Trichoderma* on plant height under green house and field conditions for two consecutive years 2010 & 2011

Sl. no	Treatment	Greenhouse trial		Field trial	
		Plant ht. (cm)* 60 DAP	% increase in ht.	Plant ht. (cm) 90 DAP	% increase in ht.
1.	IE 62	58.76± 2.28	3.01	64.5± 2.05	2.48
2.	IE 62+ T 80	59.9± 1.19	4.84	69.0± 2.12	8.84
3.	Control	57.0± 4.04	-	62.9± 1.17	-
LSD (P= 0.05)		1.23		4.26	

*Mean of three replications; DAP- Days after planting

Table.5 Stem rot incidence in two months old rice plant (cv. KD) under greenhouse and field conditions after treatment with *P. fluorescens* IE 62 and *T. inhamatum* T 80

Sl.no.	Treatment	Stem rot incidence (%)	
		Greenhouse	Field
1.	IE 62	37.1± 2.0	-
2.	S. o + IE 62	61.97± 0.99	48.28± 1.51
3.	S.o + IE 62 + T 80	43.95± 2.07	40.47± 1.26
4.	Control (S. o)	72± 1.75	100± 0
LSD (P= 0.05)		0.002	0.084

Fig.1 Antagonistic potential of A. *P. fluorescens* IE 62 against *S. oryzae*, B. *Trichoderma* T 80 against *S. oryzae*, C. *P. fluorescens* 103 against *S. oryzae* and D. Compatibility of *P. fluorescens* IE 62 and *Trichoderma* T 80

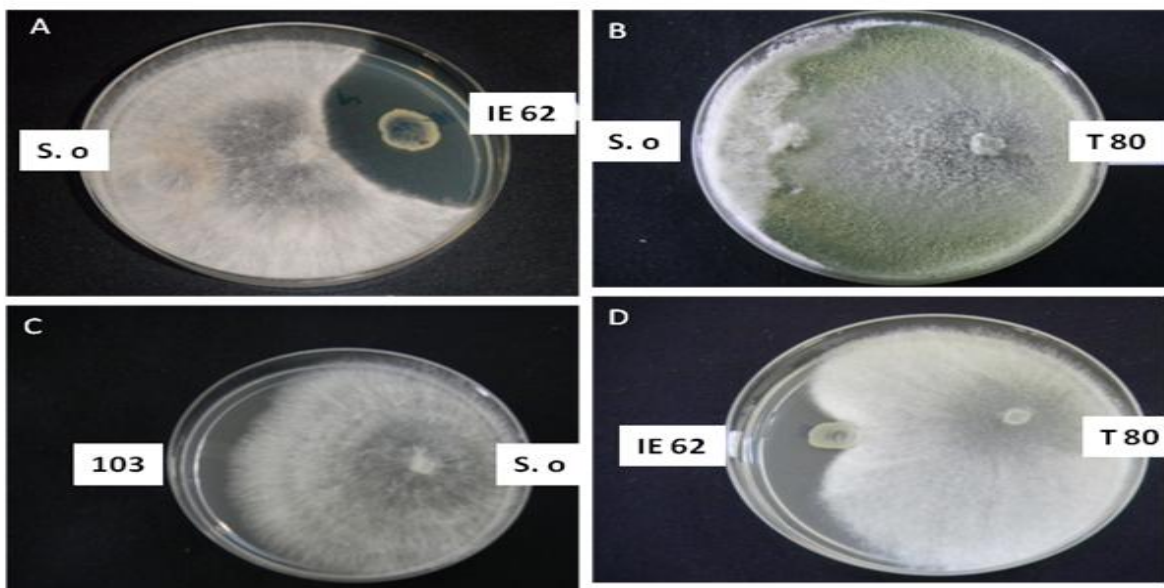


Fig.2 Effect of single and combined application of *P. fluorescens* IE 62 and *Trichoderma* T 80 on lesion formation of *S. oryzae* under greenhouse conditions

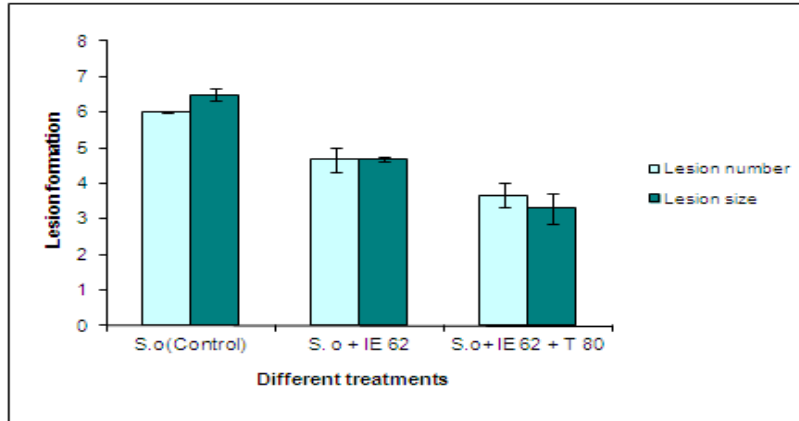


Fig.3 Effect of single and combined application of *P. fluorescens* IE 62 and *Trichoderma* T 80 on lesion formation of *S. oryzae* under field conditions

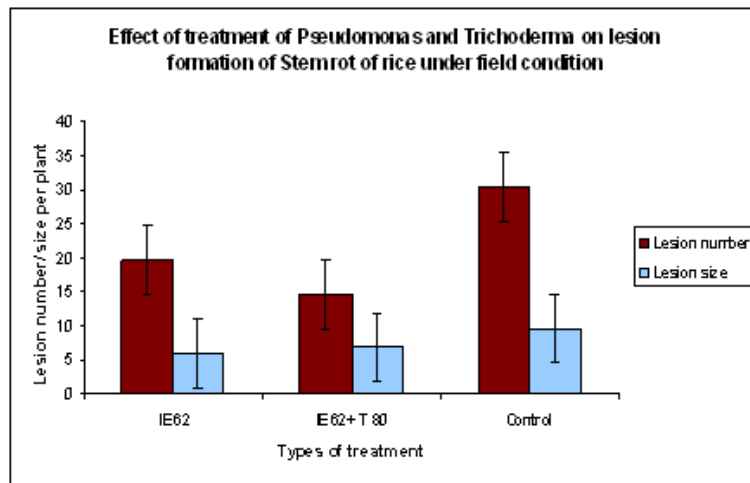
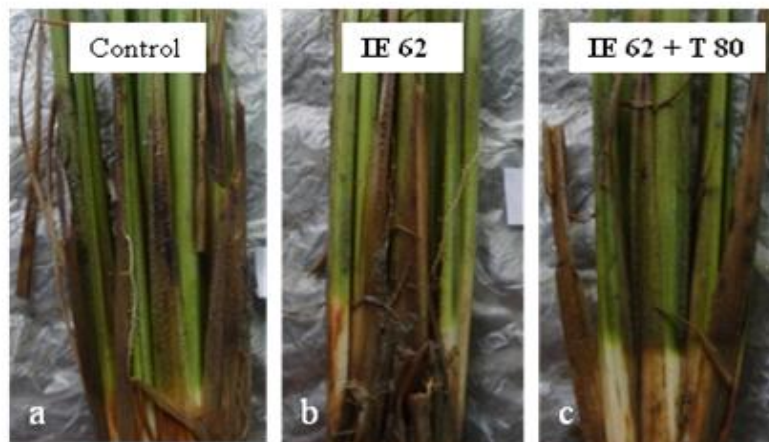


Fig.4 Stem rot infected rice plant collected from a. Control plot, b. *P. fluorescens* IE 62 treated plot and c. *P. fluorescens* IE 62 and *Trichoderma* T 80 treated plot



These growth promotion activities could be due to secretion of plant growth-promoting substances and plant growth hormones by the rhizosphere micro-organisms as demonstrated by Ureta *et al.*, 1995). The growth promotion in rice was high following the combined application of *P. fluorescens* and *T. viride* indicating that synergism existed between the two beneficial micro-organisms as shown for the combination of other micro-organisms (Camprubi *et al.*, 1995). Application of *P. fluorescens* and *T. viride* significantly reduced the sheath blight disease incidence with maximum reduction in disease severity with combined application of *P. fluorescens* and *T. viride*. Our results support the earlier observations that a combination of biocontrol agents with different mechanisms of disease control will have an additive effect and results in enhanced disease control as compared to their individual application (Guetsky *et al.*, 2002).

In conclusion, the present study identified local strains, *P. fluorescens* IE62 and *T.inhamatum* T80 that could control Stem rot of rice in Manipur and can be easily integrated into the existing production practices. Biocontrol ability may be due to well adaptation to the existing environment as revealed by field data and also due to pathogen specificity and location specific. Moreover better biocontrol efficacy was exhibited when applied in consortia in greenhouse and field trial which indicated better prospect for field applications. Development of biological control product based on these strains needs further research on repeated trail in field to study the best formulation and to ensure success of the control mechanism of the isolated rhizosphere organisms.

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