*Int.J.Curr.Microbiol.App.Sci* (2017) *6*(6): 1619-1634

*International Journal of Current Microbiology and Applied Sciences*
***ISSN: 2319-7706* Volume 6 Number 6 (2017) pp. 1619-1634**
Journal homepage: http://www.ijcmas.com

**Original Research Article** https://doi.org/10.20546/ijcmas.2017.606.190

# Blast of Rice in Manipur and its Biocontrol by *Pseudomonas fluorescens* and *Trichoderma* sp.

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**Keywords**

*Pseudomonas fluorescens*, *Trichoderma* spp., Blast of rice, Biocontrol, *Pyricularia oryzae*,

**Article Info**

***Accepted:***
21 April 2017
***Available Online:***
10 May 2017

**A B S T R A C T**

Blast of rice (*Oryza sativae* L.) caused by *Pyricularia oryzae* is one of the most destructive disease in Manipur giving losses ranging from 60 to 100%. In the present investigation, biocontrol of this disease was attempted by isolating local strains of *Pseudomonas fluorescens* and *Trichoderma* spp. *P. fluorescens* B 24 gave maximum mycellial inhibition (77.5%) among the bacterial biocontrol and *T. koningiopsis* T 162 gave maximum inhibition of 46.25%. Seed germination, root and shoot length were enhanced by single treatment (B 24) *in vitro* conditions. Plant height was also increased by 5% in greenhouse and 12.02% in field trial with single application (B 24). However, greenhouse and field data revealed significant reduction in Blast incidence (5.1% and 3.4%), lesion number (35.53% and 58.72%) and size (18.86%, 16.39%) when applied in consortia (B 24+ T 162) as compared to single application (B 24) resulting in incidence of 6.7% and 3.92%, lesion number of 33.33% and 14.67%, size of 15.09% and 27.83% irrespective of greenhouse and field trial. The results indicated the effectiveness of combined application of *P. fluorescens* B 24 and *T. koningiopsis* T 162 for plant growth promotion and control of Blast of rice and therefore can be integrated for managing Blast of rice.

## Introduction

Rice blast is the most common and destructive disease in irrigated rice of both temperate and subtropical areas of East Asia (Bonman *et al.*, 1991). Internodal culm Blast of rice was found for the first time in experimental plots of Central Agricultural University, Imphal, India and in many rice-growing areas in Manipur in 1985 with severe incidence in 1986. Punshi and KD 2-6-3, varieties widely cultivated in Manipur, suffered 60-100% yield loss (Iboton, 1987). The disease attacked rice plants from anthesis on. Symptoms were numerous white empty panicles but the uppermost leaf sheath enclosing the infected internode remained healthy.

The use of multiorganisms as crop production and crop protection inputs is currently under practice in agriculture. 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 yieldsCombined 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). In recent years, more emphasis is laid on the combined use of biocontrol agents with different mechanisms for improved disease control and also to overcome the inconsistent performance of the introduced biocontrol agents. It has been suggested that combinations of biocontrol agents could be more effective in controlling soil borne pathogens than a single agent (Nelson, 2004) to get persistent control of plant pathogens. 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), stem blight of melon (Juan Zhao *et al.,* 2012) etc. In Manipur, biocontrol of rice diseases in field has not been attempted by researchers, farmers, etc. More so ever, no work has been done on the combined effect of local *Pseudomonas* and *Trichoderma* spp. on plant growth and disease control ability of rice especially in North East India. 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 Blast of rice besides screening various biocontrol mechanisms.

## Materials and Methods

### Isolation and identification of causal organism of Blast of rice and *P. fluorescens* strain from infected rice fields

Small bits of sterilized Blast infected leaf samples collected from various locations of rice fields of Manipur 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 and was compared with the reference strain, ITCC 4511 obtained from IARI, New Delhi. The causal organism was found to be *Pyricularia oryzae* as confirmed by Koch's Postulates experiment.

A total of 158 *Pseudomonas* strains were isolated from rice rhizosphere of different locations of Manipur using serial dilution method in Kings B medium. Single colonies showing characteristic fluorescens colour when exposed to UV at 365 nm were selected and sub cultured on LB broth. The identity of the bacterial isolates was confirmed by 16S rDNA sequences and BIOLOG based identification. Single colonies were cryopreserved at -80°C in 20% glycerol filter sterilized for further studies.

### Evaluation of antagonistic potential of local *P. fluorescens* and *Trichoderma* strains against isolated culture of *P. oryzae*

All the *P. fluorescens* strains isolated from rice rhizosphere were screened for their antagonistic action against the newly isolated fungus, *P. oryzae*. In dual culture assay, the bacteria were streaked as a line on one edge of PDA (pH 6.1) in a 9 cm diameter Petri plate. After 24 h of incubation at 30º C, a 6 mm disc of an actively growing culture of *P. oryzae* was inoculated at the centre. Plates inoculated with *P. 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 (T1, T 22, T 80, T 83 and T162) with proven biocontrol potential (Kamala and Indira, 2011) collected from different ecological niches of Manipur were screened for their antagonistic potential against *P. oryzae*.

Mycelial discs of 6 mm diameter from actively growing cultures of *Trichoderma spp.* and *P. oryzae* 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 *P. oryzae* alone were treated as control. The above experiments were repeated with three replications.

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

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

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## 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 28ºC (Berg *et al.,* 2002).

Chitinase activity was tested on chitin minimal medium according to the method of Chernin *et al.,* (1995). Clearing zones indicating the enzymatic degradation were measured after1-7 days of incubation.

## Siderophore production

Siderophore was assayed by plate method using Ternary complex chrome azurol S (CAS), Fe 3+/ Hexadicyltrimethyl ammonium bromide (HDTMA) as an indicator (Schwyn and Neilands,1987). Siderophore production was indicated by the formation of a bright zone with a yellowish fluorescens in the dark blue medium.

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

*Trichoderma* isolate T162 that showed maximum mycellial inhibition of *P. oryzae* was selected for checking compatibility with *P. fluorescens* isolate B 24. For this, a small portion from the single colony of B 24 was inoculated on one edges of the PDA plate. After one day, 6 mm disc of 7 days old mycelium of *T. koningiopsis* T 162 were inoculated on the opposite side of the inoculated *P. fluorescens* isolate and incubated for seven days.

*Trichoderma koningiopsis* T 162 which could grow independently with the *P. fluorescens* isolate B 24in the plate was selected for checking combined effectivity in greenhouse and field conditions. Both the isolates were found compatible.

## Combined effect of *Pseudomonas* and *Trichoderma* on root and shoot growth of rice cv. KD in phytochamber

Rice var. KD was used as a test crop for observing seed germination, root and shoot length *in vitro* conditions. Seed treatment with talc formulation of *P. fluorescens* B 24 (33.7x $10^9$cfu/ g) and *T. koningiopsis* T 162 (1x $10^6$conidia/ ml) was done in the laboratory in aseptic condition following standard method and incubated in a growth chamber at 28±2ºC.

Seeds soaked in Luria Bertani broth served as control. The types of treatment were *P. fluorescens* B 24, ii) *P. fluorescens* B 24 + *T. koningiopsis* T 162 and iii) Control. Germination rate, root and shoot length were recorded after 5-6 days of treatment. Vigor index was calculated using the formula:

Vigour index= Percent germination x seedling length (shoot length + root length) (Abdul and Anderson, 1973).

### Screening for plant growth promotion and disease control ability in green house conditions

Pot experiment was laid out in a complete randomized design (CRD) with three replications (pots), three plants per pot in greenhouse. Pot sizes of 25x 30cm containing mixture of FYM, sand and soil to the ratio of 1/2: 1: 2 were used for the experiment. Soil application of talc based formulation was done two days before transplantation of germinated rice seedlings. 15 g of the formulation was added to the pot (size- 25x 30cm) containing mixture of FYM, sand and soil to the ratio of 1/2: 1: 2. The formulation was mixed thoroughly with the soil for uniform distribution. The pathogen inoculum was prepared by inoculating the culture of *P. oryzae* in autoclaved rice grains for 7- 10 days at 25- 28ºC. The colonized grains were used for inoculating the pathogen in the soil mixture. The treatments were given as follows: i) *P. fluorescens*–B 24 (soil application and root dip treatment), ii) *P. oryzae* (P.o) (soil application), iii) P.o+ B 24+ T 162 (soil application + root dip treatment) and iv) Control (non-treated).

The observations included plant growth, blast incidence and lesion formation by *P. oryzae.*

### Experimental layout

Experimental plots sizes of 15 x 20 ft were laid out in a RBD at Phayeng, Imphal West District of Manipur with three replicates for each treatment. Field trial was conducted for two consecutive years. Well dried FYM at the rate of 100kg/ ha was added to the experimental plots one month ahead of transplanting rice seedlings. Rice seedlings were raised under controlled conditions at IBSD, Manipur which were transplanted to the experimental plots after 30 days by giving root dip treatment (30 mins.). The treatments given were as follows:

i) *P. fluorescens* B 24 (seed treatment + soil application + root dip treatment)
ii) *P. fluorescens* B 24 + *T. koningiopsis* T 162 (seed treatment + soil application + root dip treatment)
iii) Control (seeds soaked in LB broth)

The observations were recorded on different parameters viz. plant growth, blast incidence and lesion formation. Blast 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 against *P. oryzae* and screening of their biocontrol mechanisms

All the 158 *P. fluorescens* strain isolated from rice fields of Manipur were screened for their antifungal action against *P. oryzae* which was isolated from infected rice leaf samples. Five best isolates were selected based on maximum biocontrol potentials exhibited. B 24 gave maximum mycelium inhibition with 77.5% followed by B 28 (77.08%) and IE 271 (70.2 %) respectively (Table 1, Plate 1).

The selected five *P. fluorescens* isolates namely B 24, B 28, IE 3a, IE 133, IE271, 103(IMTECH) were screened for production

of cell wall degrading enzymes such as protease, chitinase and secondary metabolite production *i.e.* siderophore (Table 1; Plate 3). B 24 showed maximum activities of all the mechanisms tested with clearance zone of 21.5 mm (protease), 22mm (chitinase) and 15.83 mm (siderophore) respectively. Reference *P. fluorescens* strain 103 showed protease activity with clearance zone of only 5mm which was lower as compared to all the five isolates screened.

### *In vitro* antifungal activity of local *Trichoderma* isolates against *P. oryzae*

Out of the 5 IBSD *Trichoderma* isolates, *T. koningiopsis* T 162 showed the highest mycellial inhibition of *P. oryzae* (46.25%) followed by T 22 (44.17%) and T 1(43.33 %)and thus T 162 was selected for further experiments (Table 2, Plate 2).

### 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 B 24 and T 80significantly enhance the germination rate. Single application with B 24 gave 94.28% seed germination and combined application (B 24+ T 162) gave 94.20% seed germination as compared to control which recorded 82.45% seed germination (Table 3). Time of grain emergence was also recorded to see the effect of treatment of *Pseudomonas* and *Trichoderma* and the result are presented in figure 1. Grain emergence was shown earliest by *Pseudomonas* isolate B 24 treated plants which emerges within 114.33 days after sowing *i.e.,* 12 days earlier than the control plant which emerges 126 days after sowing. Grain emergence was found to be delayed in P.o infected plants by 27 days. Both single and combined application significantly increased the root and shoot length as compared to control with single treatment giving more effect. Percent increase in root length was 46.41% and shoot length recorded an increase of 10.18% with single treatment of B 24 which seems to be more effective than combined treatment with T 162 which recorded an increase in root length of 34.29% and shoot length of 8.55% respectively [Table 3; $P_{(0.05)}$= 2.18 (seed germination); 4.16 (root length) and 6.48 (shoot length)].

### Effect of treatment of *P. fluorescens* B 24and *Trichoderma* isolate T 162 on height of rice cultivar KD in greenhouse and field trial

Height of rice plant was recorded 35 days after planting under greenhouse conditions (Table 4). Plant height in greenhouse experiment was found to be significantly increased with both single (60 cm) and combined treatment (59cm) with single application recording an increase in height of 5% and combined application recording a 3.39% increase in height as compared to control which recorded height of 57 cm only (Table 4; $P_{(0.05)}$= 1.19). For further confirmation, field experiment was conducted at Imphal West district of Manipur for two consecutive years. Data represented in table no.4 is pooled data of two years. Plant height was recorded three months after planting. In this experiment, both single (B 24) and combined application (B 24+ T 16) showed significant increase in plant height with 5% and 3.39% respectively in greenhouse trial and 1.41% in field trial respectively as compared to control. (Table 4; $P_{(0.05)}$=1.23, 4.26).

### Effect of treatment of *Pseudomonas* and *Trichoderma* on lesion formation of *P. oryzae* under greenhouse and field conditions

Number and size of lesions produced by *P. oryzae* on infected leaf samples were recorded 60 days after giving secondary

infection by foliar spray method. In greenhouse trial, both single and combined treatment significantly reduced lesion number and lesion size as compared to control which is infected only with *P. oryzae* (Table 5). Combined treatment (P.o+ B 24+ T 162) showed significant difference recording 35.53% and 18.86% reduction in lesion number and size respectively when compared with single treatment with B 24 22 which recorded 33.3% and 15.09% reduction irrespective of lesion number ($P_{(0.05)}$= 1.89) and size ($P_{(0.05)}$= 1.93).

In field trial, similar observations were obtained. Infected plant samples of 60days old were collected from different treatments and number and size of lesions were recorded (Table 5). Field data indicated significant reduction in both lesion number and size by single treatment with B 24as well as combined treatment with *T. koningiopsis* (T 162) as compared to control field which was infected only with *P. oryzae* under natural conditions. Single treatment (B 24) recorded less lesion number (14.67) and lesion size (5.99mm) with % reduction of 58.72 and 27.83respectivelyand combined treatment (B 24+ T 162) recorded 19.67 lesion number and 6.94mm lesion size with 44.65% and 16.39% reduction respectively as compared to control which recorded lesion number of 35.54 and lesion size of 8.3mm and the treatments were found statistically significant.

## Effect of treatment of *Pseudomonas* (B 24) and *Trichoderma* (T 162) on Blast incidence in greenhouse and field conditions

Incidence of Blast was recorded for each treatment after 3 months of planting in greenhouse conditions. Combined application (B 24+ T 162) resulted in less disease incidence (5.1%) and single application (B 24) recorded 6.7% as compared to control which recorded blast incidence of 16.5% (Table 6). This result clearly indicates that combined application of *P. fluorescens* B 24 and *Trichoderma* T 162 gave better control of blast as compared to single application.

Similar observations were obtained from field trial with combined application recording blast incidence of 3.4% than single treatment with *P. fluorescens* B 24that recorded 3.92% as compared to control which recorded 18.43%blast incidence (Table 6, Fig. 2).

## Effect of treatment of B 24 and T 162 on grain yield under greenhouse conditions

Rice grains were harvested six months after planting to see the effect of treatment of formulations of *Pseudomonas* and *Trichoderma* on grain weight and yield. Grain weight and number of grains were recorded from five rice plants of each treatment. Among the treatments, B24 treated rice plants recorded maximum grain weight (28.73g) and grain yield (1072.6) higher than the control pots which yielded 23.13g grain weight and 871.33 grain numbers (Plate 5). Combined treatment (P.O+B 24+ T 162) gave more grain weight and grain numbers (13.02g, 492.44) as compared to single treatment with only *Pseudomonas* isolate (P.O+ B 24) which attain grain weight of 8.89g and grain numbers of 276 (Figs. 3 and 4).

The present findings identified many potential *Pseudomonas* strains from rice rhizosphereof different locations of Manipur of which many of them were antagonistic against the newly isolated pathogen *i.e. P. oryzae*. The antagonistic effect of *P. fluorescens* in nutrient medium was increased by 20% with treatment with the bacterium alone (Novotna, 1990). Screening of various biocontrol mechanisms of the bacterial isolatesagainst*P. oryzae*confirms that the bacterial isolates exhibited multiple cell wall degrading enzymes and secondary metabolites which

clearly showed their role in pathogenesis.In the present study, *P. fluorescens* isolates B 24produced protease, chitinase and siderophore, *in vitro*, which possibly have contributed for their biocontrol ability in addition to antibiotics.Production of chitinase enzyme is an important criteria as chitin is the main component of the cell wall. A positive relationship was observed between the antifungal activity of chitinolytic *P. fluorescens* isolates and their level of chitinase production (Velazhahan *et al*., 1999). Chitinase, P-1,3 gluconase and cellulase are especially important fungus controlling enzymes due to their ability to degrade the fungal cell wall components such as chitin,P1,3 glucan and glucosidic bonds (Schroth and Hancock, 1981; Chet, 1987; Lorito *et al*., 1996). *Pseudomonas fluorescens* produce chitinase which involved in lysis and fragmentation of fungal cell wall and suppression of phytopathogenic fungi (Jaharamma *et al*., 2009). Chitinase excreting microorganisms have been reported as efficient biocontrol agents (Sneh, 1981; Ordentlich *et al*., 1988). In contrast to the mycelial inhibition in dual cultures, all the six *P. fluorescens* isolates differed in their biocontrol ability possibly due to the differences in root colonization and production of antifungal metabolites in natural environments.Bacterial antagonists have twin advantage of faster multiplication and higher rhizosphere competence hence *Pseudomonas fluorescens* has been successfully used for biological control of several plant pathogens and its application as biocontrol agents has drawn wide attention because of the production of secondary metabolites such as siderophores, antibiotics, volatile compounds, HCN, enzymes and phytohormones (Weller *et al*., 2002; Nagarajkumar *et al*., 2004). Voisard *et al*., (1989) observed that supression of black rot of tobacco was due to the production of HCN by *P. fluorescens* which also induced resistance in the host plant. *Pseudomonas fluorescens* (AUPF25) produce protease, IAA and siderophore and showed inhibition of mycellial growth of *Pyricularia oryzae*, a causal organism of blast disease of rice. Antagonistic assay of *T. koningiopsis* T 162 *against P. oryzae in vitro* conditions resulted in 46.25% inhibition of mycellial growth of *P. oryzae. T. hamatum* was reported to reduce the sclerotial production and parasitized the sclerotia of *S. oryzae* (Haroon Usmani, 1980). *Trichoderma* spp. were known to penetrate and colonize both the sclerotia and mycellium of *S. rolfsii* (Henis *et al*., 1983).

Investigation on PGPR by both *Pseudomonas* B 24 and *Trichoderma* T 162 showed positive results which corroborates with the findings of Cattelan *et al.,* (1999) who concluded that *Pseudomonas* GN1201 increased significantly the dry shoot weight, root length and root dry weight in soybean crop as compared to control. Similarly, Mishra *et al.,* (2010) reported that Plant growth promoting *Pseudomonas* strains increased 27.6% productivity in *Pelargonium graveolens* L. *herit*. *Pseudomonas* MR-18 increased dry weight, height of *Mucuna pruriens* by 84 and 24% respectively (Deshwal *et al.,* 2011). All the above literatures supported that plant growth promoting *Pseudomonas* increased plant growth.

Besides promoting plant growth, both the fungal and bacterial biocontrol were found to be efficient against Blast of rice. Rajbir Singh and Sinha (2005) studied the effect of *P. fluorescens* strains 1and 5 against sheath blight, *R. solani* on rice under glasshouse conditions. They found that *P. fluorescens* of higher rate, *i.e.*, 8 g/l was highly effective in reducing disease severity (60.0%) and incidence (35.6%) and increasing grain yield (33.8%) and 1000-grain weight (12.9%).

**Table.1** Antagonistic activity of five local *P. fluorescens* isolates against *P. oryzae* and its biocontrol mechanisms in vitro conditions

| Sl.no | Bacterial isolates | Antagonistic activity against *P. oryzae* | | Biocontrol mechanisms exhibited by *P. oryzae* | | |
|---|---|---|---|---|---|---|
| | | Colony diam. (mm*) | % of growth inhibition | Protease (mm*) | Chitinase (mm*) | Siderophore (mm*) |
| **1.** | B 24 | 18 ± 1.2 | 77.5 ± 1.44 | 23±1.15 | 22±0.6 | 18±1.14 |
| **2.** | B 28 | 21± 1.0 | 77.08± 2.92 | 21.5± 0.3 | 12± 1.2 | 15.83± 0.3 |
| **3.** | IE 3a | 31.2 ± 2.5 | 61.04± 3.11 | 20.7±0.28 | 12± 1.2 | 15.2± 0.6 |
| **4.** | IE 133 | 29.42± 0.71 | 63.23 ± 0.9 | 13± 0.6 | - | - |
| **5.** | IE 271 | 23.9 | 62.07±1.1 | 21±0.28 | 13±0.9 | - |
| **6.** | 103(IMTECH) | 71.3 | 15 | - | - | - |
| **7.** | Control | 80 | 8.01 | | | |
| **LSD(P= 0.05)** | | 0.01 | 0.001 | 0.011 | 0.004 | 1.1 |

*Mean of three replicates

**Table.2** Nutritive value of ripe mango per 100g Antagonistic activity of five local *Trichoderma* isolates against *P. oryzae*in vitro conditions

| Sl. No | *Trichoderma*isolates | Antagonistic activity against *P. oryzae* | |
|---|---|---|---|
| | | Colony diam.(mm) | % of growth inhibition* |
| 1. | T1 | 45.33 ± 0.8 | 43.33 ± 1.2 |
| 2. | T22 | 44.67 ± 0.9 | 44.17 ± 1.102 |
| 3. | T61 | 55.7 ± 1.2 | 30.42 ± 1.5 |
| 4. | T83 | 52 ± 1.15 | 35 ± 1.44 |
| 5 | T162 | 43 ± 1.73 | 46.25 ± 2.16 |
| 6. | Control | 80 | 8.01 |
| LSD(P= 0.05) | | 1.59 | 1.46 |

*Mean of three replicates

**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. | Treatment | % of seed germination | Root | | Shoot | |
|---|---|---|---|---|---|---|
| | | | ***Length(mm)**** | ***Increase in length (%)*** | ***Length(mm)**** | ***Increase in length (%)*** |
| 1. | B 24 | 94.28± 0.14 | 68.3± 9.27 | 46.41 | 56± 2.31 | 10.18 |
| 2. | B 24+ T 162 | 94.20± 0.54 | 55.7± 7.45 | 34.29 | 55.0± 2.89 | 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

| Sl. no | Treatment | Greenhouse trial | | Field trial | |
|---|---|---|---|---|---|
| | | **Plant ht.(cm)* 60 DAP** | **Increase in ht. (%)** | **Plant ht.(cm)* 90 DAP** | **Increase in ht. (%)** |
| **1.** | B 24 | 60.0± 1.0 | 5 | 71.5± 3.28 | 12.02 |
| **2.** | B 24 + T 162 | 59 ± 1.73 | 3.39 | 63.8 ± 1.42 | 1.41 |
| **3.** | Control | 57.0± 4.04 | - | 62.9± 1.17 | - |
| **LSD (P= 0.05)** | | 1.23 | | 2.66 | |

* Mean of three replications, DAP- Days after planting

**Table.5** Effect of treatment of *Pseudomonas* and *Trichoderma* on formation of lesions of Blast of rice in 3 months old plant in both greenhouse and field trial

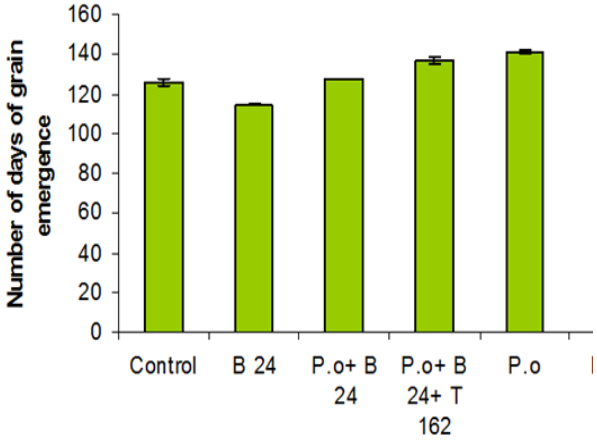
| **Sl .no** | **Treatment** | **Greenhouse condition** | | | | **Field condition** | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | **Lesion no.*** | **% reduction in lesion no.** | **Lesion size(mm)*** | **% reduction in lesion size** | **Lesion no.*** | **% reduction in lesion no.** | **Lesion size(mm)*** | **% reduction in lesion size** |
| 1. | P.o( Control) | 15.0± 1.53 | - | 5.3± 0.130 | - | 35.54± 0.53 | - | 8.3±0.61 | - |
| 2. | P.o+ B 24 | 10±0.58 | 33.33 | 4.5± 0.09 | 15.09 | 14.67±0.61 | 58.72 | 5.99±0.73 | 27.83 |
| 3. | P.o+ B 24+ T 162 | 9.67± 0.67 | 35.53 | 4.3± 0.16 | 18.86 | 19.67±0.93 | 44.65 | 6.94±0.53 | 16.39 |
| LSD(P= 0.05) | | 1.89 | | 1.93 | | 1.67 | | 1.74 | |

* Indicates mean of three replicates, P.o- *Pyricularia oryzae*, B24- *P. fluorescens* isolate, T 162- *Trichoderma* isolate

**Table.6** Blast incidence in three months old rice plant (cv. KD) under greenhouse and field conditions after treatment with *P. fluorescens* B 24 and *T. koningiopsis*T 162

| Sl. No | Treatment | Blast incidence (%)* | |
|---|---|---|---|
| | | Greenhouse | Field |
| 1. | B 24 | 6.7± 0.05 | 3.92± 0.12 |
| 2. | B 24 + T 162 | 5.1± 0.89 | 3.4 ± 0.18 |
| 3. | Control (P.o) | 16.5 | 18.43 |
| LSD (P= 0.05) | | 0.002 | 0.084 |

**Fig.1 and 2** Effect of treatment of B 24 and T 162 on grain emergence of rice plant (var. KD) under green house conditions and Blast incidence under field condition



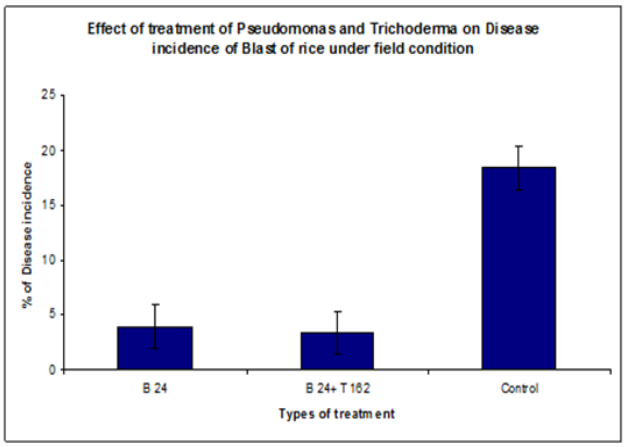
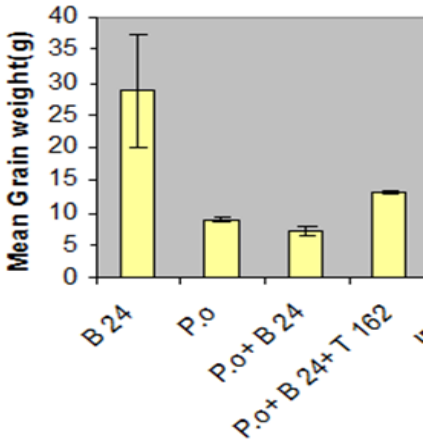




**Fig.3 and 4** Effect of treatment of B 24 and T 162 on grain yield of rice plant (var. KD) under greenhouse conditions and Effect of treatment of B 24 and T 162 on grain yield



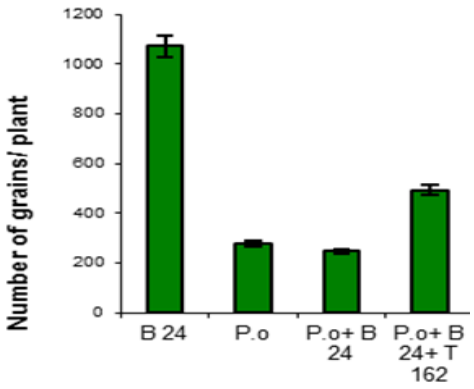
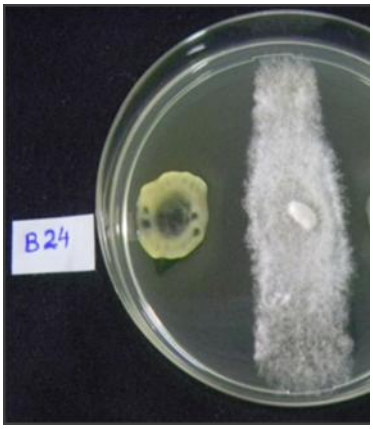



**Plate.1 and 2** *P. fluorescens* B24 inhibiting mycelia growth of *P. oryzae* and *T. koningiopsis* T162 inhibiting mycelia growth of *P. oryzae*



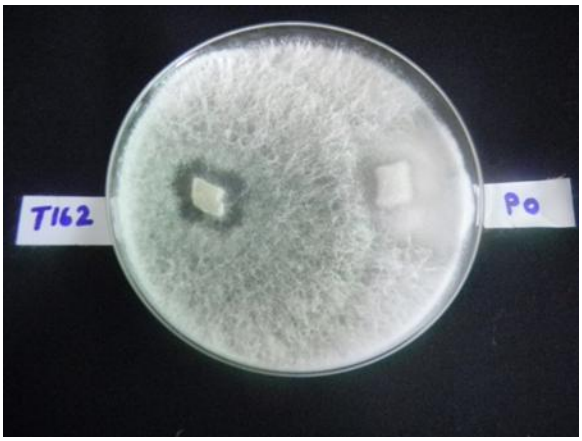
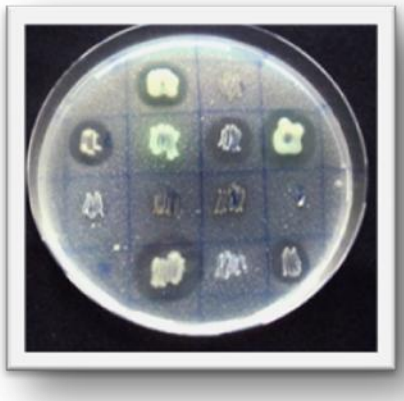
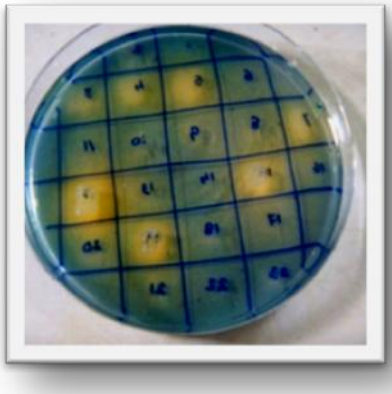




**Plate.3** Screening of a) Protease activity b) Chitinase activity and c) Siderophore production





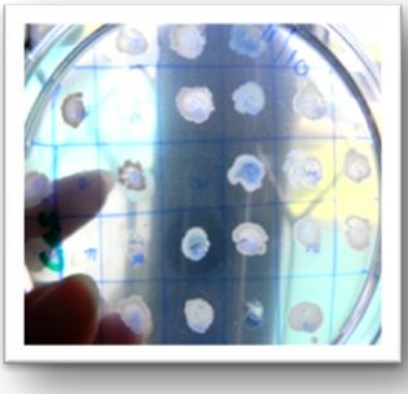


**Plate.4** Blast infected rice plant a. non treated (Control) experimental plot with numerous lesions and b. B 24 treated experimental plot showing fewer lesions

**Plate.5** Grain yield per plant from a) Control (Non- treated), b) *P. oryzae* (P.o) inoculated plant c) B 24 treated


Plant growth may have improved due to growth regulators produced by the antagonist together with their continuous supply to the developing plants as a result of the intimate contact between the seeds and the biocontrol agent (Tarek, 2002). Some *Trichoderma* strains may enhance plant growth and development (Tran, 2010). Jetiyanon and Kloepper (2002) proposed a combinational use of different biocontrol agents for improved and stable biocontrol agents against a complex of diseases. The 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). The present study identified additional biocontrol agents for control of blast of rice in Manipur which can

be easily and stably integrated into the existing production practices. Moreover field data revealed that the introduced biocontriol agent is able to adapt in the environment where it was applied as the field trial showed positive results. The newly found potential biocontrol B 24 and T 162 if provide sustained biocontrol effect in field trial on a semi commercial scale, can be extensively used for biocontrol of blast disease in rice which will be an added advantage to the control of biocontrol of many diseases of rice.

## Acknowledgement

The authors are thankful to Dr. N.C. Talukdar (the then Director in charge, IBSD, Imphal) for providing facilities and support rendered. The authors are also thankful to Department of Biotechnology, IISC, Bengaluru, for providing financial assistance to carry out this research.

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**How to cite this article:**

Subhalakshmi, T. and Indira Devi, S. 2017. Blast of Rice in Manipur and its Biocontrol by *Pseudomonas fluorescens* and *Trichoderma* sp. *Int.J.Curr.Microbiol.App.Sci.* 6(6): 1619-1634. doi: https://doi.org/10.20546/ijcmas.2017.606.190