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In vitro evaluation of bio-agents against *Pyricularia oryzae* (Cav.) causing rice blast disease

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ABSTRACT

The five different bio-agents *viz., Trichoderma viride*, *T. harzianum*, *T. virens*, *Pseudomonas fluorescens* and *Bacillus subtilis* were evaluated against *Pyricularia oryzae* at four and eight days after incubation through dual culture technique. Among the five different bio-agents, highest per cent inhibition of mycelial growth of fungus was recorded in *T. virens* i.e. 67 per cent and 70 percent after four and eight days after incubation respectively with mean of 68.5 per cent followed by *Trichoderma viride* with the inhibition of 61 and 63 per cent respectively with mean of 62 per cent. The *Pseudomonas fluorescens* did not show any inhibition of mycelial growth of *P. oryzae as* the pathogen over grew the bio-agents.

Key words: Bio-agents, Dual culture technique, *Pyricularia oryzae.*

India is the largest rice growing country accounting for about one third of the world acreage under the crop. India's annual rice production was 103.6 million tons during 2016 (Anonymous, 2016). Rice is grown in India in all the states.

Among the yield limiting factors of rice, diseases caused by different pathogens play an important role which adversely affect the yield and quality. Rice suffers from many diseases caused by fungi, bacteria, viruses, phytoplasma, nematodes and other non-parasitic disorders. Among the fungal diseases, blast is considered as a major threat to rice production because of its wide spread distribution and its destructiveness under favourable conditions. The Commonwealth Mycological Institute has recorded its presence from 85 countries throughout the world. Paddy blast is generally considered as the principal disease of rice and is caused by a fungus belonging to the Ascomycete *Pyricularia oryzae* Cavara (teleomorph= *Magnaporthe grisea* (Hebert) Barr Comb nov.). Losses due to the blast disease may range up to 90 per cent depending upon the component of the plant infected. *M. grisea* infects above ground parts of the plant, but neck blast and the panicle blast are the most damaging phases of the disease and have been shown to significantly reduce yield, grain weight and milling quality (Ghose *et al.,* 1960)

In vitro evaluation was carried out during 2015 in Plant Pathology Laboratory, University of Agricultural Sciences, GKVK, Bangalore, Karnataka, India with bio-agents listed in Table 1 through dual culture technique.

In the dual culture technique, twenty ml of sterilized and cooled PDA media was poured into sterile Petri plates. Fungal antagonists were evaluated by inoculating the pathogen at one side of Petri plate and the antagonist on the opposite side of the same plate by leaving 3-4 cm gap. But for bacterial antagonists, it was streaked in the centre of the plate after which a fungal disc was placed near it. Each treatment was replicated three times. After required period of incubation i.e. after the growth in the control plate reached 90 mm diameter, the radial growth of pathogen was measured. Per cent inhibition over control was worked out according to the equation of Vincent (1947).

$$I = \frac{(C-T)}{C} \times 100$$

Where,
I= Per cent growth inhibition
C= Growth of the fungus in control
T= Growth of the fungus in treatment

Analysis and interpretation of the experimental data was done by using completely randomized design (CRD) and Factorial CRD for laboratory studies ANOVA (Gomez and Gomez, 1984; Hosmand, 1988).

The results (Table 2) revealed that, maximum inhibition of mycelia growth (67%) was recorded in *T. virens* after four days of incubation. Other bio-agents *viz., T. viride,*

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Table 1: List of bio-agents used for *in vitro* evaluation against rice blast

Bio agents	Source / isolate
Trichoderma harzianum	Pathology Lab, UAS, Bangalore
Trichoderma viride	Pathology Lab UAS, Bangalore
Trichoderma virens	Pathology Lab UAS, Bangalore
Pseudomonas fluorescens	Pathology Lab UAS, Bangalore
Bacillus subtilis	Pathology Lab UAS, Bangalore

T. harzianum and B. subtilis recorded 61 per cent, 44 per cent and 11 per cent growth inhibition, respectively. However, *P. fluorescens* did not show any inhibition of mycelial growth of *P. oryzae as* the pathogen over grew the bio-agents.

Similarly after eight days of inoculation, *T. virens* showed highest growth inhibition (70%) followed by *T. viride* (63%).Whereas *B. subtilis* and *T. harzianum* recorded 12 per cent and 44 per cent growth inhibition respectively. *T. harzianum* did not show any variation in the per cent growth inhibition with incubation time.

Biological control is a potential non-chemical, effective and eco-friendly means for plant disease management. In the present investigation the efficacy of bio-agents against *P.oryzae* was assessed by the dual culture technique.

Among the antagonists, the maximum inhibition of *P. oryzae* was observed in *T. virens* (67%). Differential inhibition was observed in *T. viride* (61%), *T. harzianum* (44%), and *B. subtilis* (11%). Contrarily, no inhibition was observed in *P. fluorescens*. The present study is in confirmatory with the observations made by Watanabe (1985), Hajano *et al.,* (2012), Arumugam *et al.,* (2013) and Ali and Nadarajah (2014). Gouramanis (1997) observed that the *T. harzianum* inhibit 70-88 per cent of mycelial growth and conidial germination of *P. oryzae*. Gnanamanickam and Mew (1992) and Joshi and Gardener (2006) reported least inhibition by *P. fluorescens* and *B. subtilis* as against higher inhibition by *Trichoderma* spp.

In the present study all the species of *Trichoderma* tested showed more hyphal inhibition compared to bacterial antagonists. This might be due to the production of antibiotics, which are detrimental to the growth of *P. oryzae*. (Karthikeyan and Gnanamanickam, 2008) and also may be due to higher competitive ability of *Trichoderma* spp.

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Table 2: *In vitro* evaluation of bio-agents against *P. oryzae*

Bio-agents	Per cent growth inhibition (Days after incubation)		Mean (%)
	4	8	
Trichoderma viride	61.0	63.0	62.0
Trichoderma harzianum	44.0	44.0	44.0
Trichoderma virens	67.0	70.0	68.5
Pseudomonas fluorescens	0	0	0
Bacillus subtilis	11.0	12.0	11.5
SEm±	**0.62**	**0.79**	
C.D at 1%	**1.92**	**2.49**	

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