

IN VITRO STUDY ON BROWN LEAF SPOT OF RICE CAUSED BY HELMINTHOSPORIUM ORYZAE

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ABSTRACT

Leaf brown spot is a serious disease of rice causing considerable yield losses. Among various control measures the uses of chemical fungicides have been found highly effective . In vitro studies were carried out To evaluate the new fungicide, CMFF 136 WP (Propineb 54.2% + Tricyclazole 15.0%) against the brown spot of rice. To study the induction of ISR in paddy due to treatment with PGPR, fungicides and challenge inoculated with *B. oryzae*.

INTRODUCTION

Rice (*Oryza sativa* L.) is one of the major cereal crops of the world used as the staple food by 60% of the world's population (Nahar et al., 2016). The majority of the rice (90%) is being produced in Asian countries, of which China and India being the major producers (Kumar et al., 2011)

In India, brown spot occurs every year on most cultivated rice varieties. The disease especially occurs in environment where water supply is scarce combined with nutritional imbalance particularly lack of nitrogen (Baranwal

et al., 2013). At present, there are very limited strategies for the control of brown spot and cultivars with an adequate level of resistance are not available (Srinivasachary et al., 2011). Though effective synthetic fungicides are available for major diseases of rice, there is a continuing need for more effective and safer fungicides. Hence, it was thought that the new fungicide molecule viz., CMFF 136 WP (Propineb 54.2% + Tricyclazole 15.0%) developed by M/S. Coromandel International Ltd., may be tested for the management of brown leaf spot of rice.

The indiscriminate use of chemical fungicide to control the disease is not only hazardous to living being but also adversely affects the environment. This necessitates finding out alternative approaches which are economically feasible and eco-friendly.

Under such circumstances, use of Plant Growth Promoting Rhizobacteria (PGPR) offer promising means of controlling diseases and improve the yield of rice (Mew and Rosales, 1992). Of the different PGPR's, the group of bacteria viz., Fluorescent pseudomonads offer great potential as it provides effective control of the disease, induces growth promoting effects and systemic resistance. Earlier studies have also proved the effectiveness of *P. fluorescens* in reducing brown spot severity (Hass and Defago, 2005; Joshi et al., 2007). Integrating PGPR with the new fungicide could enhance the disease suppression and also reduce the amount of fungicides applied to the crop.

MATERIALS AND METHODS

Efficacy of certain antagonists against *B. oryzae*

Among the antagonists tested, *P. fluorescens* was found to be more antagonistic to *B. oryzae* as it recorded the maximum per cent inhibition of the mycelial growth, mycelia dry weight and conidial germination. In the present study, the mycelial growth and mycelia dry weight of *B. oryzae* was found reduced with an increase in the concentration of culture filtrates of all the isolates.

Effect of combination of *P. fluorescens* and CMFF 136(Propineb 54.2% + Tricyclazole 15.0%) for the management of brown leaf spot of rice caused by *B. oryzae*.

In the present study, rice crop treated with *P. fluorescens* ST @ 10.0 ml/kg of seeds + 2% foliar spray at 45 DAT+ CMFF 136 as foliar spray at @ 0.1% conc. on 60 DAT recorded minimum brown spot incidence and also recorded improved biometrics and yield of rice when tested both under pot culture and field conditions. Hence, when the antagonist was combined with a new combination chemical viz., CMFF 136, greater suppression of the disease incidence and significant increase in the yield component of rice was also noticed. Also it was possible to reduce the amount of chemical being used and also to reduce the number of applications viz., with only one spray of the chemical at 60 DAT, the brown spot incidence could effectively be managed with enhanced crop yield.

RESULT AND DISCUSSION

Table 6: Effect of culture filtrate of *P. fluorescens* on the mycelial growth and mycelial dry weight of *B.oryzae*

Tr. No.	Conc. of the culture filtrate (%)	Solid medium assay		Liquid medium assay	
		Mycelial growth (mm)	Per cent Inhibition (%)	Mycelial dry weight (mg)	Per cent Inhibition (%)
1	5	36.63	59.3	159.27	46.95
2	10	29.31	67.43	99.00	67.02
3	15	19.43	78.41	45.74	84.75
4	20	9.8	89.11	20.19	93.28
6	40	NG	-	0.84	99.72
7	Mancozeb 75 % WP (0.1%)	NG	-	1.00	99.67
8	Untreated Control	90.0	-	300.25	-
	SE	0.92		0.25	
	CD (p=0.05)	2.12	--	0.68	--

NG – Nil Growth

Table 8: Effect of fungicide CMFF 136 WP (Propineb 54.2% + Tricyclazole 15.0%) on the mycelial growth and mycelial dry weight of *B. oryzae* (In vitro)

Tr. No.	Conc. of the fungicide (ppm)	Solid medium assay		Liquid medium assay	
		Mycelial growth (mm)	Per cent inhibition (%)	Mycelial dry weight (mg)	Per cent Inhibition (%)
1	100	34.68	61.46	205.98	30.77
2	200	32.67	63.70	162.72	45.32
3	300	29.34	67.40	102.69	65.49
4	500	11.41	87.32	47.75	83.95
5	1000	NG	100.00	1.24	99.58
6	1200	NG	100.00	0.85	99.71
7	Mancozeb 75 % @ 0.1%	NG	100.00	1.15	99.61
8	Control	90.00	--	297.57	--
	SE	0.37	--	1.12	--
	CD (p=0.05)	1.04	--	2.26	--

NG - Nil growth

**Table 9: Evaluation of CMFF 136 WP (Propineb 54.2% + Tricyclazole 15.0%)
against *B. oryzae* under in vitro condition**

Tr. No.	Conc. of the fungicide (ppm)	Inhibition Zone (mm)	
		Paper disc assay	Agar well method
1	100	7.12	7.85
2	200	11.23	8.23
3	300	14.56	11.80
4	500	15.65	16.20
5	1000	16.25	17.31
6	1200	17.37	17.78
7	Mancozeb 75% WP 0.1%	15.23	16.12
8	Control	90.00	90.00
	SE	0.15	0.04
	CD (p=0.05)	0.34	0.07

The fungistatic activity of *P. fluorescens* based on the inhibition of mycelial growth and mycelia dry weight of several pathogens was well established (Balabaskar, 2006; Sundaramurthy et al., 2014). In the present study, the culture filtrate of *P. fluorescens* at 40 per cent conc. recorded complete inhibition of conidial germination of *R. solani*. As observed in the present study, several workers have reported about the inhibitory effect *P. fluorescens* on the conidial/sclerotial germination of pathogens (Rangeshwaran and Prasad, 2000; Umamaheswari et al., 2002; Rajamohan, 2014). The variability in the pathogenicity among isolates of *B. oryzae* was reported by several workers (Lee et al., 1984; Prasad and Bharat, 1995; Harish et al., 2007; Peeyush Kumar et al., 2011). Vijayakumar (1998) also mentioned that the variability in virulence among five isolates of *B. oryzae* in rice. The variations in brown spot incidence in different locations could well be attributed to the difference in virulence of the *B. oryzae* isolates. The results of present study are in agreement with these earlier reports.

Further, from the present study, it was discernible that the rice crop between the age of 75 -80 DAT showed more incidence of brown spot which could be due the fact that, the crop/ variety is susceptible during the maximum tillering stage. A similar observation was made by Misra (1973) with regard to *B. oryzae* infection in rice. Similar to the present observations high incidence of brown spot was observed on cultivar PR116 in sub-mountainous region of the Punjab state during 2000 to 2005 by Hunjan et al. (2007). Also, it is noteworthy that the isolates which produced faster mycelial growth and more conidia were highly virulent and produced higher brown spot incidence which could be due the virulence of the pathogenic isolates.

Thus, the results of the present study have clearly proved that with the combination of *P.fluorescens* along with the new chemical CMFF 136 WP (Propineb 54.2% + Tricyclazole 15.0%) it is possible to reduce the amount of chemical being used and also to reduce the number of applications viz., with only one spray of the chemical at 60 DAT the brown spot incidence could effectively be managed with enhanced crop yield.

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