J. Mycopathol. Res. ***52(2)*** *: 351-354, 2014;* (ISSN 0971-3719)

Efficacy of biocides in the management of Brown leaf spot (*Drechslera oryzae)* of paddy (*Oryza sativa* L.)

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Received : 20.11.2013 RMS Accepted : 08.07.2014 Published : 27.10.2014

Effects of six different biocides were evaluated to control Brown leaf spot pathogen (*Drechslera oryzae*) of paddy in glass house conditions. In order to evaluate the antagonistic potential of *Trichoderma harzianum* (Kanpur) was superior to *T.viride* (Kanpur), *T.harzianum* (Rajasthan), *T.viride* (Rajasthan), bioformulation of *T.harzianum* and *T.viride* which produced 39.55 per-cent growth inhibition in dual culture technique. Pre-application of spore suspension of biocides hampered Brown leaf spot lesion formation and protected considerable leaf area from infection showing reduction in leaf spot from 14.56 to 3.15 mean lesions per leaf. Spore suspension of *T. harzianum* (Kan.) @ 10^3-10^5 conidia/ml gave maximum per cent disease control 79.06, whereas minimum per cent disease control 50.03 was recorded with (Kan.) @ 10^3-10^5 conidia/ml.

Key words: Antagonist, biocides, Brown leaf spot, foliar spray, paddy

INTRODUCTION

Drechslera oryzae (Breda de hann), Subramanium and Jain, causal organism of the Brown leaf spot disease of paddy is prevalent all over the world. Chauhan (2002) has reported that the yield loss due to this disease has been estimated to be around 0.15 million tonnes annually in eastern part of India. The use of resistant variety cannot provide long lasting protection, because it may become susceptible due to leaching down of K, Fe and Mn (Singh, 2005) and also influence for development of resistant strain from the pathogen. On the other hand, chemical control of plant disease is not considered economic and ecofriendly. Biocontrol of the plant pathogens have received increasing attention as a promising supplement to chemical control (Baker, 1989). Bioagents (i.e. *Trichoderma* sp., *Aspergillus* sp., *Chaetomium* sp., *Bacillus* sp., *Pseudomonas* sp., *Agrobacterium* sp. etc.) have been exploited for their biocontrol potential against several plant pathogenic fungi (Prasad *et al.,* 1978; Barnett and Lilly, 1982; Mostafa, 1993; Mandal, 1995; Biswas, 1997; Sen, 1999; Singh, 2005). Bioagents represent the most promising antagonistic microorganism against several plant pathogens. Hence, efficacy of biocides is undertaken in the present investigation for management of Brown leaf spot of rice disease.

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MATERIALS AND METHODS

The variety Pant-12 is a popular variety of rice commonly used by the farmers at Kanpur, Uttar Pradesh and this genotype has been proved susceptible to Brown leaf spot. The experiment was conducted using two isolates of *T. harzianum,* two isolates of *T. viride* and two bioformulations of *T.*

harzianum and *T. viride.* Among these two isolates of *T. harzianum* and *T. viride,* one of each was collected from Rajasthan and rest from Kanpur and designated as *T. harzianum* (Raj.), *T. viride* (Raj.) *T. harzianum* (Kan.) and *T. viride* (Kan.). The cultures were revived time to time and stored at 4°C in a refrigerator.

In vitro efficacy of biocontrol agents against Drechslera oryzae through dual culture plate technique

The biocontrol potential of used antagonists was determined by dual culture techniques (Dennis and Webster 1971). Disc (5 mm dia) of seven days old culture of *D. Oryzae* were placed in a sterilized Petriplate poured with 20 ml PDA at 1 point leaving 1 cm distance from the periphery of one side. Opposite side 1 cm distance from the periphery of plate a disc of seven days old culture of *T. harzianum* (Raj.), *T. harzianum* (Kan.), *T. viride* (Raj.) and *T. viride* (Kan.) was placed separately. Antagonistic activity of bioformulation of *T. harzianum* , bioformulation of *T. viride* was done by Agar well method. A well of 5 mm diameter was made at one point leaving 1 cm distance from the periphery of one side which was filled by solution of each bioformulation and on the opposite site disc (5 mm dia.) of *D. Oryzae* were placed in separately. One plate was kept without antagonist to serve as control. Observations on the growth of pathogen were taken by measuring length and width of colony at one corner, after 7 days of incubation at 25± 1°C. In addition to colony growth, inhibition zone was also measured after a week. PGI was calculated according to following formula Per cent Growth Inhibition = C-T/C X 100 (C= Growth of mycelium in control; T= Growth of mycelium in treatment)

Effect of biocides on disease development

A pot experiment was conducted for two consecutive years at glasshouse to evaluate the efficacy of foliar spray of biocides against *D. oryzae*. One month old seedlings with 5-6 leaf stage were given inoculation treatment with spore suspension of bioagent and solution of bioformulation and virulent strains of *D. oryzae.* Two sets of control plants where seedling were sprayed only conidial suspension of *D. oryzae* and with water. Disease observations were recorded after seven days of inoculation on formation of Brown leaf spot by using five point scale (0-IV) (Nayak and Padmanabhan, 1970). Leaves with no sign of infection received a score of Zero while those with highest infection (i.e with the 76 or above leaf blighted) received a score of IV. Similarly, leaves with 1-25, 26-50 and 51-75, area covered with Brown spot lesions received a score of I, II, III respectively. The per cent disease index (PDI) was calculated by the following formula. Percent Disease Control (PDC) = PDI in check – PDI in treatment/ PDI in check X 100

$$\text{Per cent Disease Index (PDI)} = \frac{\Sigma \text{ Class rating} \times \text{Class frequency}}{\text{Total no. of leaves} \times \text{maximum class rating}} \times 100$$

RESULTS AND DISCUSSION

The present investigations were undertaken to evaluate the biocide viz. four bioagents (two isolates of *T. harzianum,* two isolates of *T. viride)* and two bioformulations of *T. harzianum* and *T. viride* with the goal of reducing the incidence of *Drechslera oryzae* as well as stimulating the growth of paddy seedlings.

In vitro efficacy of biocontrol agents against Drechslera oryzae through dual culture plate technique

In order to evaluate the antagonistic potential of bioformulation of *T. viride* T_1, *T. harzianum* (Kanpur) T_2, *T. harzianum* (Rajasthan) T_3, bioformulation of *T. harzinum* T_4, *T. viride* (Rajasthan) T_5, *T. viride* (Kanpur) T_6 were tested against *Drechslera oryzae* T_7 in dual culture, and significant suppression of mycelial growth of host pathogen was noted (Table1). *T. harzianum* (Kanpur) T_2 was superior to *T. viride* (Kanpur) T_6, *T. harzianum* (Rajasthan) T_3, *T. viride* (Rajasthan) T_5, bioformulation of *T. harzinum* T_4 and bioformulation of *T. viride* T_1 which produced 39.55 per cent growth inhibition in dual culture technique. The inhibitory effect of *T. harzianum* (Rajasthan) T_3, *T. viride* (Rajasthan) T_5 on radial mycelia growth of *D. oryzae* was recorded and there was no significant difference between the two. The mycelial growth of *D. oryzae* in dual culture was reduced up to 39.55% as compared to single culture of fungus after seven days of incubation. Due to reduction of mycelial growth of *D. oryzae* production of certain metabolites and hypersensitive response by bioagents (Srinivas and Ramakrishnan 2003). Butler (1958) observed inhibitory effect of *Tricho-*

Table1 : Antagonistic effect of biocides on mycelial growth of *Drechslera oryzae* under *in vitro* condition

Treatment	Mean radial growth of mycelium(mm)*	Percentage growth inhibition*
Bioformulation of *T. viride* T_1	46.0	17.87 (25.37)**
T. harzianum (Kan.) T_2	33.8	39.55 (39.24)
T. harzianum (Raj.) T_3	37.0	33.84 (35.86)
Bioformulation of *T. harzianum* T_4	40.7	27.33 (31.83)
T. viride (Raj.) T_5	37.5	32.94 (35.31)
T. viride (Kan.) T_6	36.6	34.55 (36.28)
D. oryzae (check-I) T_7	56.0	0.00 (4.05)
S.Em±	1.28	1.46
C.D (P=0.05)	2.78	3.16

*Average of three replications
** Figures in the parentheses are angular transformed (X+0.5) values.

derma sp. on the growth of *Cochliobolus sativus,* prefect stage of *Drechslera sorokiniana.* Prasad *et al.* (1978) also reported that *T. viride* antagonized the spot blotch pathogen. The culture filtrate of *Chaetomium globosum* antagonized the conidia of *Drechslera sorokiniana* causal agent of spot blotch of wheat and also stimulated the plant growth (Biswas, 2001). From these results, it was emphasized that the culture filtrate of bioagents probably contains some kind of antifungal metabolites and growth promoting substances.

Effect of foliar spray of biocides on disease severity of brown leaf spot of paddy

The data presented in Table 2 showed that all the treatments were significantly effective in minimizing disease severity. The spore suspension of different bioagents when applied as foliar spray before inoculation with *D. oryzae* restricted the formation of Brown leaf spot lesion on paddy. The susceptible rice variety Pant-12 developed on an

Table 2 : Effect of biocides against *Drechslera oryzae* under glass house conditions

Treatment	Dose	Mean lesions per leaf (No.)*	Per cent Disease Index (PDI)*	Per cent reduction compared to control (Check-I)*
Bioformulation of *T. viride* T_1	4 g / Liter	4.36 (2.19)**	19.47 (26.53)***	67.14 (55.33)***
T. harzianum (Kan.) T_2	10^3-10^5 conidia/ml	3.15 (1.90)	12.40 (21.04)	79.06 (63.13)
T. harzianum (Raj.) T_3	10^3-10^5 conidia/ml	3.27 (1.93)	14.20 (22.53)	75.96 (60.99)
Bioformulation of *T. harzianum* T_4	4 g/Liter	5.24 (2.38)	27.95 (32.23)	52.74 (46.86)
T. viride (Raj.) T_5	10^3-10^5 conidia/ml	4.42 (2.21)	21.24 (27.78)	64.03 (53.46)
T. viride (Kan.) T_6	10^3-10^5 conidia/ml	5.38 (2.42)	29.52 (33.21)	50.03 (45.30)
D. oryzae (check-I) T_7	10^3-10^5 conidia/ml	14.56 (3.87)	59.21 (50.60)	0.00 (4.05)
Healthy (Check-II) T_8	-	0.00 (0.70)	0.00 (4.05)	100.00 (90.00)
S.Em±		0.13	0.84	1.30
C.D (P=0.05)		0.29	1.79	2.79

*Average of three replications
**Figures in the parentheses are square root transformed (X+0.5) values.
***Figures in the parentheses are angular transformed (X+0.5) values.

average 14.56 lesions/leaf whereas numbers of Brown leaf spot lesions were reduced significantly in all the treatments. Significant differences in disease control varied from 79.06 to 50.03% between treated and untreated plants. The maximum disease control (79.06%) and lowest disease index (12.40%) were recorded in *T. harzianum* (Kan.) T_2 treated plants whereas in case of *T. harzianum* (Raj.) T_3 treated plants the respective values were 75.96% and 14.20%. In case of disease plants (Check-I) T_7 average number of lesion per leaf was 14.56 and disease index become as high as 59.21%. The plant treated with bioformulation of T. *harzianum* (T_4 *and* bioformulation of T. *viride)* T_1 were not found as good as suspension of bioagents because here disease index and disease control was 27.95%, 29.52% and 52.74%, 50.03% respectively. The increasing disease control from 0.00 to 79.06 % and declining disease index from 59.21% to 12.40% could be possibly due to use of bioagents. Thus, the protection provided by inoculation with bioagents as foliar sprays indicated that resistance was expressed by decrease in the disease severity. The effect of *T. harzianum* on fungal pathogen infecting wheat and barley showed that the antagonist resulted in reduction in the incidence of *Cochliobolous sativus* (Fernandez, 1992). Seed treatment with a *P. fluorescens* PGPR strain led to reductions in the numbers of foliar lesion caused by subsequent inoculation of *P.s.* pv. *phaseolicola* (Alstrom, 1991).

The present investigation revealed that pre-inoculation with biocides is one of the best strategies to manage brown spot of paddy.

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