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# Effect of Bioagent Application on Progress of Bacterial Leaf Blight of Rice, Under Glasshouse Conditions

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***Abstract* - Present study was carried out to evaluate different bioagent formulations against bacterial leaf blight disease of rice by using different epidemiological parameters and compared with check and chemical treatment, under glasshouse conditions. Disease progress curves were developed for different treatments and infection rate and area under disease progress curve was calculated. Disease progress curves of bacterial leaf blight disease revealed that bioagent formulations were found to be effective in reducing the progress of disease as compared to control. After 19 days of application, bioagents formulations were found to be significantly effective than chemical treatment in reducing progress of disease. Infection rate of disease recorded with bioagent formulations was lower than check and chemical treatment. Application of bioagents resulted significantly reduced the area under disease progress curve as compared to check and chemical treatment and increased grain yield was recorded.**

***Keywords* - Area Under Disease Progress Curve, Infection Rate, *Pseudomonas* Spp., *Trichoderma* Spp.**

## I. Introduction

Bacterial leaf blight of rice caused by *Xanthomonas oryzae* pv. *oryzae* is the disease of great economic importance in all the rice growing areas of the world and is particularly destructive in South East Asia including India (Mew *et al*., 1993) during heavy rains of monsoon season. The disease has caused enormous losses in India. A combination of metrological factors such as high temperature, high humidity, heavy rain fall, high light intensity and frequent typhoons favored the out break of the disease (Murlidharan and Venkatarao, 1979). Bioagents are potential candidates for management of plant diseases as they offer several advantages over chemical control like, (A.) There are no pollution problems and health hazards, (B.) It is more stable without any development of resistance in pathogen, (C.) Biological control agents are non-phytotoxic, (D.) It may have growth promoting effect and (E.) It causes little disturbance in ecological balance. The microbial antagonists occur in nature, they are host specific, virulent, self perpetuating and genetically stable. *Trichoderma* spp. and *Pseudomonas* spp. are exhibits inhibitory effect on *X. oryzae* pv. *oryzae* (Gangwar and Sinha, 2010a&b; Gangwar and Sinha, 2012a) and decrease bacterial leaf blight of rice (Gangwar, 2012; Gangwar and Sinha, 2012b).

Many workers (Adhikari *et al*., 1994; Ahmed *et al*., 1997; Oña *et al*., 1998) developed disease progress curve (DPC), calculated infection rate (IR) and area under disease progress curve (AUDPC) for bacterial leaf blight of rice. Disease progress curves exhibit possible disease incidence and yield loss. Pattern of DPC, IR and AUDPC depict about the epidemic on set and the reaction by host plant. Mew *et al.* (1993) studied disease progress of bacterial leaf blight related to stages of plant growth into seedbed, seedlings, panicle initiation, flowering and mature grain stages. Effectiveness of bioagents against different plant diseases was reported by several workers (Elmer and McGovern, 2004; Verma and Dohroo, 2005; Daghman *et al*., 2006) by the assessment of infection rate and AUDPC. In the present study, effectiveness of bioagent formulations against bacterial leaf blight of rice was tested by using different epidemiological parameters such disease progress curve, infection rate and area under disease progress curve. These epidemiological parameters help to compare the effectiveness of bioagent formulations and chemicals at different periods of time.

## II. Materials and Methods

### *Glasshouse Experiment*

Experiment was conducted in the glasshouse of Department of Plant Pathology, G. B. Pant University of Agriculture and Technology, Pantnagar. Nursery was raised in field plots at Crop Research Center. Experiment was carried out using susceptible rice cultivar Jaya. Plastic pots were filled with natural field soil and fertilized with NPK (@ 120:60:40 kg/ha). Pots were puddle before transplanting of rice seedlings. Two seedlings (21 days old) of rice were transplanted in each pot. Pots were regularly watered and three replications were maintained for each treatment.

### *Mass multiplication of fungal and bacterial bioagents*

*Trichoderma harzianum* was mass multiplied on barnyard millet (*Echinocloa frumentacae*). Grains colonized by *Trichoderma* were air dried in open shade and ground with the help of Willy Mill to get fine powder. This powder was passed through 50 and 80 mesh sieves simultaneously to obtain spore powder. Whereas, *P. fluorescens* was mass multiplied on King's B broth. Both spore powder and broth culture diluted with talcum powder (mesh = 350 with 95% whiteness) and 1% carboxyl methyl cellulose (CMC) to get desired concentration ($10^6$ cfu/g) of bioagents in the formulation.

### *Fungal and bacterial bioagent formulations*

Influence of two *Pseudomonas fluorescens* formulations (*Pf* 83 and PBA-2), two *Trichoderma harzianum* formulations (*T. harzianum* and PBA-1) and one mixed formulation of *P. fluorescens* + *T. harzianum* formulation (PBA-3) on disease progress curve of bacterial leaf blight of rice were tested along with chemical treatment [0.03 g streptocycline + 1 g copper oxychloride per liter water)] and check. Bioagent formulations ($10^6$ cfu/g) were applied @10 gram per liter water.

*Application of treatments*

Pathogen was inoculated by clipping off the leaf tip @ $10^6$ cell/ml inoculum (Kauffman *et al*. 1973). Bioagents and chemical treatment were applied next day of pathogen inoculation and check pots were treated with sterilized water. Pots were arranged in randomized block design. Successive application of treatments was given after 7 days after first application. Under glasshouse, minimum and maximum temperature was 28 and 35 $^0$C, relative humidity was 80-90 %, and photoperiod was 14 hours. Data on percent disease severity recorded 7 days after first spray at 3 days interval. Grain yield was recorded after harvesting.

*Calculation for infection rate*

Disease progress curves were developed by plotting disease severity (%) against time. Apparent infection rate was calculated by recording disease severity at 3 days interval by using following formula (Vanderplank, 1963):

$$r = \frac{2.3}{t_2 - t_1} \log 10 \frac{x_2(1-x_1)}{x_1(1-x_2)}$$

where,

$x_1$ = Disease index at time $t_1$(time of first disease rating)

$x_2$ = Disease index at time $t_2$ (time of second disease rating)

r = Apparent infection rate

Disease severity (%) was divided with 100 (for converting into unit x) and used in place of disease index in the above formula for calculating apparent infection rate of bacterial leaf blight of rice.

*Calculation for infection rate*

Area under disease progress curve (AUDPC) was calculated by using following formula (Shanner and Finney, 1977):

$$\text{AUDPC} = \frac{\left(\frac{D_1+D_2}{2}\times T\right)+\left(\frac{D_2+D_3}{2}\times T\right)\ldots\ldots\left(\frac{D_{n-1}+D_n}{2}\times T\right)}{n-1}$$

where,

D = Percent disease severity at different time intervals ($D_1$ $D_2$ $D_3$........ $D_n$)

T = Time interval (days) between two observations

n = Total number of observations

*Statistical analysis*

Statistical analysis of the data obtained from experiment was done at the computer centre of G. B. Pant University of Agriculture and Technology, Pantnagar, using appropriate programme as per the requirement of the experiment. The critical difference (CD) was calculated at 5% level of significance for comparison of difference between the means of different treatments.

## III. Results and Discussion

*Effect of bioagent formulations on disease severity and disease progress curve*

Bacterial leaf blight disease severity recorded with bioagent formulations and chemical treatment was significantly lower than check after 7, 10, 13 and 16 day of application. At 19, 21, 24 and 27 days after treatment application, all bioagent formulations exhibited significantly lower disease severity as compared to check and chemical treatment (Table 1). Minimum mean disease severity (21 %) was recorded with application of PBA-2, PBA-3 and *T. harzianum* which was followed by PBA-1 and *Pf* 83 exhibited 24 % disease severity. Disease Progress Curve (DPC) developed for different treatments exhibited that untreated check depicted short lag phase because of quick population buildup. This is followed by prolonged log phase because pathogen attacked plant tissues without any restriction. Short decline phase was observed because no more healthy tissues were available for further infection. DPCs for bioagents (Fig. 1) showed short lag and log phase which were not distinct and prolonged decline phase.

Table 1: Effect of bioagent formulations on bacterial leaf blight disease severity at different days after first treatment application, under glasshouse conditions

| **Treatments** | **Disease severity (%)** | | | | | | | | **Mean** |
|---|---|---|---|---|---|---|---|---|---|
| | **7 DAT** | **10 DAT** | **13 DAT** | **16 DAT** | **19 DAT** | **21 DAT** | **24 DAT** | **27 DAT** | |
| ***Pf* 83** | 3 | 18 | 26 | 27 | 28 | 29 | 30 | 31 | 24 |
| **PBA-2** | 9 | 19 | 25 | 27 | 29 | 31 | 33 | 35 | 21 |
| ***T. harzianum*** | 4 | 15 | 20 | 23 | 25 | 26 | 27 | 29 | 21 |
| **PBA-1** | 7 | 17 | 26 | 26 | 27 | 28 | 29 | 31 | 24 |
| **PBA-3** | 9 | 15 | 20 | 22 | 23 | 24 | 25 | 26 | 21 |
| **Chemical Treatment** | 5 | 12 | 16 | 21 | 35 | 48 | 60 | 80 | 35 |
| **Check** | 16 | 28 | 48 | 69 | 88 | 97 | 100 | 100 | 68 |
| **Mean** | 8 | 18 | 26 | 31 | 36 | 40 | 43 | 47 | 31 |
| **CD at 5 %** | A (days after treatment application) = 2<br>B (treatments) = 2<br>A × B = 4 | | | | | | | | |

* Mean of three replications; DAT = Days after first treatment application

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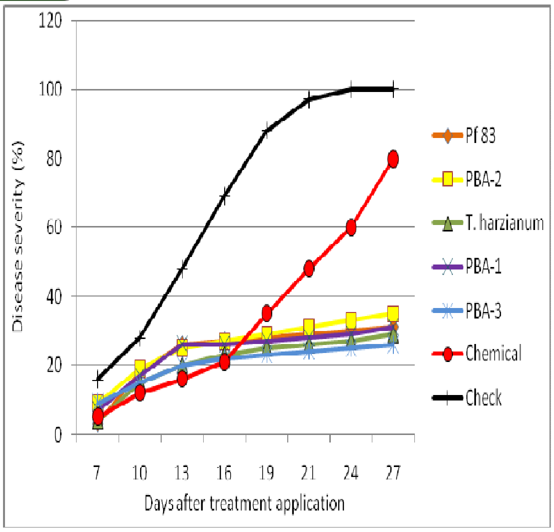

Fig.1. Effect of different bioagent formulations and chemical treatment on progress of bacterial leaf blight disease of rice, under glasshouse conditions

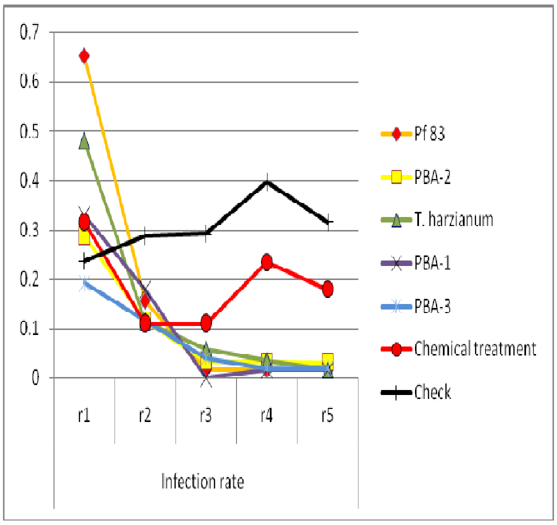

Fig.2. Effect of different bioagent formulations and chemical treatment on infection rate of bacterial leaf blight disease of rice, under glasshouse conditions

Such pattern of DPCs clearly indicates that bioagents did not work at the initial stage of disease development and allow to pathogen to buildup population quickly hence showed short lag phase. Short log phase may also be due to the fact that bioagents started to work and affected population growth of pathogen, as a result log phase changed to decline phase. There is small increase in disease severity hence prolonged decline phase was observed. Whereas in the case of chemical treatment, prolonged lag and log phase were observed and there was no decline phase. This indicates that chemical treatment reduced the initial population of the pathogen and has adverse effect on further growth of pathogen so prolonged lag and log phase was observed. Absence of decline phase indicates that chemical treatment does not have prolonged effect on pathogen. After 7, 10, 13 and 16 day of application, chemical treatment was exhibited significantly higher effectivity against bacterial leaf blight as compared to bioagent formulations. Whereas at 19, 21, 24 and 27 days after application, all bioagent formulations took over and showed significantly higher effectivity against bacterial leaf blight over chemical treatment. Monaco *et al.* (1999) calculated disease progress curve, AUDPC and the epidemic rate with several saprobic fungi (*Nigrospora* spp., *Penicillium* spp., *Chaetomium globosum*, *Cladosporium cladosporioides* and *Trichoderma polysporum*) which inhabited *Alternaria solani* in tomato phylloplane.

*Effect of bioagent formulations on infection rate*

Apparent infection rates were calculated for progress of the disease for different time interval. All treatments showed significantly higher infection rate ($r_1$) in early stage of disease progress as compared to check except PBA-3 which is statistically equal to check. However, all treatments exhibited significantly lowered infection rate ($r_2$) at later stage of disease progress as compared to check. All bioagents showed significantly lower $r_3$, $r_4$ and $r_5$ as compared to check and chemical treatment (Fig. 2).

This indicated that bioagents are effective in lowering apparent infection rate for longer duration in later stage of disease development. However, chemical treatment was effective in lowering apparent infection rate in early stage of disease development. Effectiveness of bioagents against different plant diseases was reported by several workers (Elmer and McGovern, 2004; Verma and Dohroo, 2005 and Daghman *et al.*, 2006) by the assessment of infection rate and AUDPC. Minimum apparent infection rate, decreased AUDPC and increased seed germination was observed by Verma and Dohroo (2005) against *Fusarium* wilt of pea with the bioagents*, T. viride* and *T. harzianum* as compared to control.

*Effect of bioagent formulations on area under disease progress curve*

Based on disease incidence expressed as the AUDPC, Daghman *et al.* (2006) concluded that the *T. harzianum* (UPM40) dry preparation was effective in protecting the seeds and seedlings against pre and post emergence damping-off caused by *Rhizoctonia solani* in leaf mustard (*Brassica rapa*). It is evident from Figure 3 that all bioagent formulations showed significantly lowered AUDPC as compared to check and chemical treatment. However, maximum reduction in AUDPC was observed for PBAT-3 (70.47%) which is followed by *T. harzianum* (69.02%).

The results indicate that bioagents are effective in reducing amount of disease. Elmer and McGovern (2004) also reported that bioagents exhibited significant reductions in the AUDPC which indicate the suppression of *Fusarium* wilt of cyclamen. Effectiveness of *Pseudomonas chlororaphis* 63-28 and *Bacillus cereus* HY06 against *Pythium* root rot of chrysanthemum was estimated by Liu *et al.* (2001) based on percent roots colonized by *Pythium* and AUDPC. They observed reduced values of AUDPC by 61 and 65% in plants treated with *P. chlororaphis* 63-28 and *B. cereus* HY06. For *P. dissotocum*, the respective strains reduced AUDPC values by 70 and 90%. Present study revealed that all bioagent formulations were exhibited significantly higher grain yield as compared to check and chemical treatment. Maximum increase in grain yield (67.65%) was observed with *T. harzianum*, followed by PBA-1 (66.47%) and PBA-2 (65.29%).

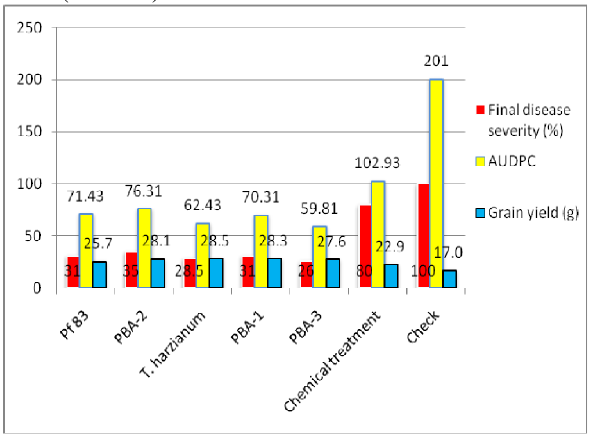


Fig.3. Effect of bioagent formulations and chemical treatment on area under disease progress curve (AUDPC) and grain yield per plant (g), under glasshouse conditions

Present study revealed that all bioagent formulations were found to be effective against bacterial leaf blight of rice. Significantly reduced disease severity and infection rate over chemical treatment was recorded after 19 days of bioagent application. Significantly reduced the area under disease progress curve and increased grain yield was recorded with bioagent application. Long lasting effect on disease was recorded with bioagent application in the present study, under glasshouse conditions. Further studies are considered necessary in this area of research.

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## Author's Profile

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My major field of M.Sc. Thesis Research was studies on mass multiplication and compatibility of *Beauveria bassiana* (Balsamo) Vuilemin with chemical and bio-pesticides and of Ph. D. Thesis Research was biological management of bacterial leaf blight of rice by caused by *Xanthomonas oryzae* pv. *oryzae* (Uyeda and Ishiyama) Dowson. I have eight research papers published in the journals of repute. Presently, I am working as research associate.
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*My academic has listed down*

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| Ph. D. | 2010 | Plant Pathology | GBPUA&T, Pantnagar |
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