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# EVALUATION OF NATIVE BIOCONTROL AGENTS FROM RICE FIELDS OF ASSAM

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**Abstract–** The present investigation was carried out to observe the efficacy of native biocontrol agents isolated from rice fields of Assam. Results revealed that native *Pseudomonas flurescens* were more efficient as compared to native *Bacillus* and *Trichoderma* sp. against Bacterial blight pathogen, *Xanthomonas oryzae* when applied singly. The highest inhibition of bacterial blight pathogen was recorded in the consortia of *T. viride + P. fluorescens + B. subtilis* with 68.56 % inhibition followed by 59.84% in treatment of *P. fluorescens* alone.

## INTRODUCTION

The importance of sustainable agriculture is becoming one of the vital issues of the present scenario of agricultural development and research throughout the world. In this scenario of improved agriculture, pest and diseases still continue to play a major limiting role in enhanced crop production. During the past couple of decades, the use of plant growth promoting rhizobacteria (PGPR) for sustainable agriculture has increased tremendously in various parts of the world. It has been shown that selected rhizobacterial population enhances plant growth and yield in addition to suppression of phytopathogens (Glick, 1994; Timmusk and Wagner, 1999). The use of these rhizobial microorganisms with the aim of improving nutrients availability for plants is also an important practice and necessary for agriculture (Figueiredo *et al.,* 2010). One of the dominant PGPR under the order Pseudomonds, *Pseudomonas fluorescens* acts as an active biocontrol agent of soilborne diseases on a wide range of crop plants. Rice is highly vulnerable at all stages of growth to different pathogens that affect the quality and quantity of its yield. Among the diseases bacterial blight (BB) caused by *Xanthomonas oryzae* pv. *oryzae*, is one of the most important and oldest known diseases of rice. Soil chemical and physical properties have been implicated in the variable biocontrol activity of *P. fluorescens* and *Trichoderma koningii* against take-all disease of wheat and results indicated that introduced biocontrol agents and indigenous populations of antagonistic microbes are influenced by some abiotic soil conditions. *P. fluorescens* isolated from rice rhizosphere is naturally suppressive to bacterial blight caused by *X. oryzae* pv. *oryzae* (Sharma and Bora, 2016; Jeyalakshmi *et al.,* 2010) but level of control may depend on the status of soil micronutrient, predominant type of clay mineral, their effect on the pathogen or host or host pathogen interactions. Therefore, there has been a burning need to find out alternative methods by exploiting microbial antagonist to reduce the inoculum potentials in their active or dormant state.

## MATERIALS AND METHODS

Soil samples from Rice rhizosphere along with roots were collected in bulk from different locations of Jorhat district of Assam. The root samples along with adhering soil particles were separated from the bulk soil for the isolation of rhizosphere bacteria.

The isolated cultures were confirmed as *Pseudomonas fluoresecns, Bacillus subtilis* and *Trichoderma viride.* through their morphological and biochemical studies.

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The inhibitory effect of different biocontrol agents *viz*., *P. fluorescens Bacillus subtilis* and *Trichoderma viride* isolates were tested *in vitro* against Xoo adopting dual culture assay technique of Aspiras and Cruz (1985) with few modifications. Following treatment combinations were compared using NA and KB as basal media:

i. Growth of *X. oryzae* pv. *oryzae* in presence of different biocontrol agents *viz*., *P. fluorescens*, *Bacillus subtilis* and *Trichoderma viride* isolates
ii. Control = Growth of *X. oryzae* pv. *oryzae*

To evaluate the inhibitory effect of *P. fluorescens*, *Bacillus subtilis* and *Trichoderma viride* against Xoo, assay plates of Xoo were prepared by seedling the bacterium in NA media. For these, 48 h growth of Xoo was washed with sterile distilled water to prepare the stock culture of Xoo. Serial dilution of stock bacterial suspension was made upto $10^{-8}$ level. From the final dilution 0.1ml aliquot was pipetted asceptically to petriplates, each containing approximately 15ml NA medium separately and allowed to solidify. Similarly cultural plates of *P. fluorescens* and *Bacillus subtilis* were prepared by seeding with the 0.1ml aliquot of the bacterial suspension in NA medium and *Trichoderma viride* in PDA medium. After 48 h of growth, a 0.5 cm diameter growth of *P. fluorescens*, *Bacillus subtilis* and *Trichoderma viride* in NA and PDA was scooped out with the help of a sterilized cork borer and transferred to the centre of NA plates seeded earlier with cells of Xoo. Plates were then incubated at 28±1°C and after 2 days of incubation, the inhibition zones were recorded for each treatment. The presence of a clear zone of inhibition surrounding the disc was indicative of inhibitory (antimicrobial) activity against Xoo. The inhibition percentages of Xoo were calculated by using the formula described by Mayee and Datar (1986) as,

$$\text{Percent inhibition} = \frac{\text{Mean of inhibition zone (mm)}}{90} \times 100$$

## RESULTS AND DISCUSSION

The antagonistic potential of isolated *P. fluorescens*, *Bacillus subtilis*. and *Trichoderma* sp were tested against Xoo adopting dual culture method using nutrient agar (NA) as basal media. Culture of Xoo grown alone in NA medium served as control. The inhibition zones produced (mm) or overcrowding shown by the native isolates against Xoo in NA media were recorded and tabulated (Table 1).

**Table 1.** Suppressive effect of different antagonist and their consortia against *Xanthomonas oryzae pv. oryzae* isolated from rice rhizosphere

| Treatments | | Bioagents Inhibition (%) |
|---|---|---|
| $T_0$ | *X. oryzae pv. oryzae* | 0.00 (0.57) * |
| $T_1$ | *P. fluorescens* | 59.84 (50.65) |
| $T_2$ | *B. thuringiensis* | 55.15 (47.93) |
| $T_3$ | *T. viride* | 32.26 (34.76) |
| $T_4$ | *T. viride + P. fluorescens* | 42.09 (40.40) |
| $T_5$ | *T. viride + B. thuringiensis* | 39.24 (38.76) |
| $T_6$ | *P. fluorescens + B. thuringiensis* | 46.92 (43.22) |
| $T_7$ | *T. viride + P. fluorescens + B. thuringiensis* | 68.56 (55.86)* |
| | S.Ed (±) | 1.67 |
| | $CD_{0.05}$ | 2.87 |

*Data in the parenthesis are angular transformed values

Data presented in Table 1 revealed that all the native isolates produced varying sizes of inhibition zones against Xoo *in vitro*. The inhibition produced by the consortia of *T. viride + P. fluorescens + B. thuringiensis* was significantly highest (68.56 %) followed by *P. fluorescens* (59.84%) and *B. thuringiensis* (55.15 %). Least inhibition was shown by *T. viride* (32.26 %).

## CONCLUSION

In the present investigation, we studied the *in vitro* efficacy of different native isolates of *T. viride + P. fluorescens + B. subtilis* isolated from rice rhizosphere as an effective biocontrol strategy along with better plant growth and soil health for management approach of bacterial blight of rice and *vis-a-vis* for cultivation of organic rice in Assam.

However, more studies should be emphasized under farmers' field condition for determination their efficacy in disease management along with better plant growth and soil health and the subsequent monetary benefits the farmers will achieve by using these native bio control agents. Moreover, there is need to develop a single complete package for overall management of other serious problems of rice and increase yield. This technology should be transferred to field condition and demonstration programme should be conducted in the farmer's field in order to spread the technology amongst farming communities. These efforts, if successful will be a very fruitful approach towards production of organic rice in Assam.

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