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Assessment of efficacy of microbes for control of bacterial blight disease of rice

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ABSTRACT

The bacterial blight disease is known to occur in epidemic proportions in many parts of the world, incurring severe crop loss of up to 50%. Crop loss assessment studies have revealed that this disease reduces grain yield to varying levels, depending on the stage of the crop, degree of cultivar susceptibility and to a great extent, the conduciveness of the environment in which it occurs. The severity and significance of damages caused by infection have necessitated the development of strategies to control and manage the disease, so as to reduce crop loss and to avert an epidemic. Keeping in view of the importance of bacterial blight disease the present study was under taken to isolate individual bacteria from a naturally occurring bio product and to evaluate and assess the efficacy of microbes for control of bacterial blight disease of rice.

Key words: Bacterial blight disease, microbes, rice.

INTRODUCTION

Rice is perhaps the most widely cultivated food crop world over and it has a special benefit as it has eight of essential amino acid in delicately balanced proportion [1].Rice production is constrained by diseases of fungal, bacterial and viral origin.

Bacterial blight (BB) of rice, caused by *Xanthomonas oryzae* pv. Oryzae (Xoo) is one of the oldest known diseases and subsequently, its incidence has been reported from different parts of Asia, northern Australia, Africa and USA[2]. Blight disease is a vascular disease resulting in a systemic infection that produces tannish-grey to white lesions along the veins[3]. Symptoms are observed at the tillering stage, disease incidence increases with plant growth, peaking at the flowering stage[3-4]. The bacterial blight disease is known to occur in epidemic proportions in many parts of the world, incurring severe crop loss of up to 50%[5]. The severity and significance of damages caused by infection have necessitated the development of strategies to control and manage the disease, so as to reduce crop loss and to avert an epidemic. Though the use of Bordeaux mixture, antibiotics and other copper and mercurial compounds were resorted to in the early fifties, environmentally safe and stable chemical control agents rendering control at very low concentrations are yet to be developed[6]. Today, the exploitation of host resistance appears to be the only reliable method of disease management [7].The other novel way is to use antagonistic organisms to kill a positive agent [8].The present investigation is to identify antagonistic bacteria against *Xanthomonas Oryzae.* Pr. Oriyazae for eco-friendly control of the pathogen.

MATERIAL AND METHORDS

Method of sterilization

Moist heat sterilization was done by autoclave for sterilization of glass wares such as beaker, test tubes, patriplates and conical flasks at 121° C at 15 PSI pressure for 15 minutes. Dry heat sterilization was done by hot air oven at different temperature for sterilization. Sprit lamp is used for sterilization, nicrome ware loop and for creating sterilizing zone.

Preparation of Different media

Different media were used for culturing microorganism by maintaining suitable condition. Potato Sucrose Agar media, Potato Dextrose Agar media and Peptone Glycerol Agar media were prepared at suitable conditions and sterilized by pouring these solution into conical flasks by raping them properly.

Plate preparation

Petri plates, Test tubes and other glasswares were rapped properly and autoclaved. After that all Autoclaved glassware's were dried properly by hot air oven. Glassware's then kept inside laminar flow for 15 minutes treating with UV light. Before transferring the media from conical flasks to Petri plate's hands were washed with 70% alcohol. Under laminar floor beside sterile zone of sprite lamp media were transferred to patriplates and allow them to cool. After cooling the media the patriplates were inverted to avoid moisture.

Isolation of Bacteria from Bio product:

Serial dilutions of bi product were done for detection of different bacteria by following method.

1:10 dilution

2 µl of sample were added with 18 µl of distilled water. From these above solution 10 µl were taken and 500 µl (1% agar) were added.

1:100 dilutions

2 µl of sample were added with 198 µl of distilled water. From these above solution 10 µl were taken and 500 µl (1% agar) were added.

1:1000 dilutions

5 µl of sample were added with 5ml. of

Table 1: Percentage of infection recorded

	Date09.01.2007		Date15. 1. 2007		Date21.1. 2007		Date26.01.2007		Date31.01.2007	
	Total	Mean	Total	Mean	Total	Mean	Total	Mean	Total	Mean
Isolates 1	103.81	12.97625	110.51	13.8137	162.02	20.2525	195.54	24.4425	242.97	30.37125
Isolates 2	115.72	14.465	140.62	17.5775	179.68	22.46	213.55	26.6937	235.83	29.47875
Isolates 3	161.14	20.1425	179.23	22.4037	189.39	23.6737	194.05	24.2562	223.06	27.8825
Isolates 4	167.42	20.9275	181.46	22.6825	195.15	24.3937	208.36	26.045	216.01	27.00125
Isolates 5	126.21	15.77625	144.84	18.105	154.95	19.3687	162.07	20.2587	171.23	21.40375
Control	150.81	18.85125	267.92	3.49	355.42	44.4275	466.15	58.2687	554.57	69.32125

distilled water. From these above solution 10 µl were taken and 50 µl (1% agar) were added.

1:10,000 dilutions

10 µl of solution were taken from 1:1000 dilutions then 90 µl of distilled water were added from this 10 µl of sample were taken and 500 µl (1% agar) were added.

Maintenances of Mother Culture

Mother Cultures were maintained properly with suitable media like P.S.A for different isolates. Pure mother cultures were maintained inside test tube and Petri plates. These tubes or plates were wrapped properly by the help of cotton and Para film. After proper wrapping these mother cultures were kept inside incubator at 300 C for 24 hours for micro-Isolates growth properly. After complete growth of microbes these mother cultures were transferred to refrigerator for storing.

In vitro test against B.L.B

Individual isolates were taken from mother culture and allow them to grow in Petri plates by serial streaking and kept inside incubator. Bacteria *Xanthomonas* was also cultured in Petri plates by serial streaking method. Each individual Isolate was cultured along with *Xanthomonas* simultaneously by different streaking method to observe their growth rate and action. *Xanthomonas* was cultured one day after with each individual isolates by parallel streaking. Individual isolates were also cultured among themselves by parallel streaking to observe their different mechanism and action like antagonistic effect. *Xanthomonas* was placed in the middle of the plate and other isolates were placed to the surrounding of the plate to observe the growth rate.

***In vivo* test against B.L.B**

Different bacteria were cultured in patriplates in suitable condition. Little amount of sterile water were added to patriplates carefully. Bacterial spores were brushed up carefully and transferred to glass beaker. Few amount of distilled water were added to the above solution to prepare final bacterial solution. These solutions were transferred into a test tube for proper mixing of the solution with the help of a shaker.

Inoculation of Rice plant and Disease assessment

The pot of rice plants were labeled with the isolates to be inoculated. Pair of sterile scissors was dipped into the inoculums suspension. Leaves were clipped 1-2 cm from the tip with the pair of scissors infested with bacteria.

Different clipped diseases leaves were measured by its disease length, lesion type after 14 days of inoculation. The disease reaction of the test cultivars to the 5 races.

RESULTS

By in vitro testing, antagonistic effects of bio product bacteria's were found in Petri plates among with *Xanthomonas* by parallel streaking.

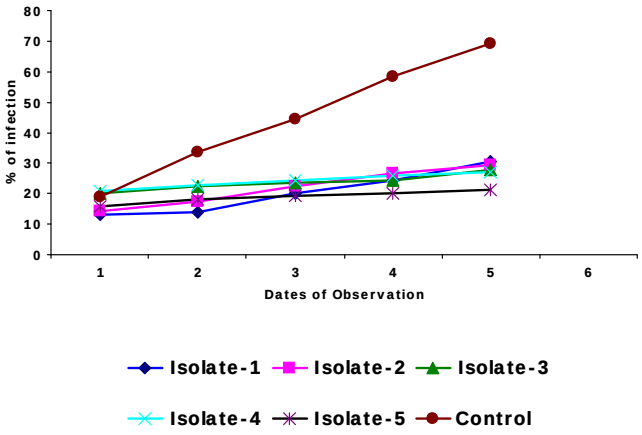

Fig. 1: Disease progress curve of five isolates

Different isolates produce their reaction against *xanthomonas* in Petri plates and able to inhibit the growth of *xanthomonas* in *in-vitro* condition. After getting antagonistic effect of bio-products isolates in vitro condition, in vivo testing was done by inoculating rice plant in net house following results were observed between test and control rice plant statistically by taking 8 disease replication of 5 different isolates and 1 control in 5 different dates. The disease progress curve of five isolates and results are given in Fig. 1 and Table 1 .

DISCUSSION

Crop loss assessment studies have revealed that bacterial blight disease reduces grain yield to varying levels, depending on the stage of the crop, degree of cultivar susceptibility and to a great extent, the conduciveness of the environment in which it occurs . The severity and significance of damages caused by infection have necessitated the development of strategies to control and manage the disease, so as to reduce crop loss and to avert an epidemic. Though the use of Bordeaux mixture, antibiotics and other copper and mercurial compounds were resorted to in the early fifties, environmentally safe and stable chemical control agents rendering control at very low concentrations are yet to be developed. Today, the exploitation of host resistance appears to be the only reliable method of disease management. Bacterial blight disease management can reduce the initial inoculums and subsequent development of the pathogen on host plants and this can be accomplished through chemical protection, host plant resistance, and biological control. Biological control can be done by isolating antagonistic bacteria which can completely overcome the BLB.

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