EVALUATION OF FOLIAR SPRAYING OF *BACILLUS SUBTILIS* AND *ACHROMOBACTER XYLOSOXIDANS* FOR MANAGEMENT OF BACTERIAL LEAF BLIGHT (BLB) OF RICE UNDER FIELD CONDITION

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ABSTRACT

Akhtar, S., Sultana, A., Shupta, S. A., Chakraborty, S. and Khokon, M.A.R. 2020. Evaluation of foliar spraying of *Bacillus subtilis* and *Achromobacter xylosoxidans* for management of bacterial leaf blight (BLB) of rice under field condition. Bangladesh J. Plant Pathol. 36(1&2):39-48

The research works were aimed to investigate the efficacy of beneficial rhizopheric bacteria *viz*. *Bacillus subtilis* and *Achromobacter xylosoxidans* and their culture filtrates for management of bacterial leaf blight (BLB) of rice in field condition. *In-vitro* antagonism ability of different strains of *B. subtilis* and *A. xylosoxidans* were assayed against *Xanthomonas oryzae* pv. *oryzae* following agar diffusion method. Both suspension and culture filtrates of nine *B. subtilis* strains and one *A. xylosoxidans* showed growth suppression against *X.oryzae* pv. *oryzae* at different extent. Cell culture suspension of different bacterial strains showed higher antagonism than their respective cell-free culture filtrates. *Bacillus subtilis* [BSL-26(iii)] showed highest growth (30.00 mm) at 24 Hours Post Inoculation (HPI). Based on the *in-vitro* assay, BSL-26(iii) and *A. xylosoxidans* were assayed in the field under natural epiphytotic condition of BLB and compared with bactroban. In most cases, cell suspension @ 10^8 CFU/ml of *B. subtilis* showed superior performances compared to untreated control. Significant reductions (12.64 % and 22.37 % over control) (average) in disease incidence and severity were also observed respectively when cell suspension @ 10^8 CFU/ml of *B. subtilis* sprayed on the foliage. So, this study unveiled the opportunity to utilize *B. subtilis* for field management of BLB of rice. Further, *B. subtilis* [BSL-26(iii)] can be a potential candidate for developing formulation at commercial level.

Key Words: Bacterial leaf blight, Rhizophere bacteria, Antagonism, Disease incidence, Disease severity, Management, Rice.

INTRODUCTION

Rice (*Oryza sativa* L.) belongs to family Poaceae and is one of the world's most important primary food sources for half of the people of the world (Zhu and Wu 2008). More than 90 % of the world's rice is grown and consumed in Asia (Khush 2005). There are many abiotic and biotic factors responsible for reduction of yield of rice. Among the biotic factors bacterial leaf blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* is one of the important diseases of rice (Mansfield *et al.* 2012). BLB occurs at all the growth stages of rice and is manifested by either leaf blight or "Kresek" symptoms. Bacterial blight disease is known to occur in epidemic proportions in many parts of the world, incurring severe crop loss of up to 50 % (Bala 2014).

The earlier studies have identified some chemicals and antibiotics with relative efficacy against the disease (Reissing *et al*. 1986). Repeated use of the chemical pesticides is a major concern of developing pesticide-resistant strains of the plant pathogens, destroying bio-diversity and human health hazards (Gill and Garg 2014). Application of rhizopheric bacteria can be a novel management strategy for harnessing the incidence and severity of BLB of rice instead of antibiotics. Beneficial rhizopheric bacteria have shown enormous potential to induce resistance in plants against different pathogens (Jetiyanon and Kloepper 2002). Rhizopheric bacteria also capable of bring about disease suppression by various modes of action such as antagonism, competition for space and nutrients and induction of systemic resistance (ISR) (Noumavo *et al*. 2016). For instance, *Bacillus* strain D13, which is antagonistic to *X. oryzae*

pv. *oryzae* emit volatile compounds that reduce the colony diameter and cell motility of *X. oryzae* pv. *oryzae* cultured in divided petri plates (Xie *et al.* 2018). The cell free extract of *B. subtilis* which contain cyano-compounds, suppressed the growth of all phytopathogens, especially *M. phaseolina* (Alamri *et al*. 2012). Antibiotics from *B. subtilis* like surfactin, iturin and fengycin were strongly antagonistic to some important phyto- and post-harvest pathogens (Chen *et al*. 2008). Siderophores of *A. xylosoxidans* can act in biocontrol as a determinant of induced systemic resistance in the plant (Vaidya *et al.* 2001, Forchetti *et al.* 2007). Zhang *et al.* (2016) found that the culture filtrate of two bacterial strains *A. xylosoxidans* (09X01) and *Bacillus cereus* (09B18) caused high mortality of the second stage juvenile nematodes and reduced *in vitro* egg hatch compared to control.

To our knowledge field control of BLB is yet to be dependent on selecting tolerant varieties, spraying Cu containing fungicides and some cultural practices. Moreover, application of antibiotics is not judicious and has enormous chance to develop resistant strains of bacteria. Therefore, the present study was undertaken for screening of potential antagonists to control bacterial leaf blight (BLB) caused by *X. oryzae* pv. *oryzae* for assessing the effect of foliar application of rhizopheric beneficial bacterial cell suspension (*B. subtilis* and *A. xylosoxidans*) and their culture filtrates for environmentally safe management of Bacterial Leaf Blight (BLB) of rice in field condition.

MATERIALS AND METHODS

Laboratory experiment

The *In-vitro* experiment was carried out at the laboratory of Bioactive Compounds, Bio-formulation and Bio-signaling, Plant Pathology Department, Bangladesh Agricultural University, Mymensingh.

Isolation and identification of *X. oryzae* pv. *oryzae*

Infected rice leaves showing typical bacterial blight symptoms were collected, cleaned, cut about 2 to 4 cm and sterilized with 1 % sodium hypochlorite solution for 30 seconds, then washed in sterilized distilled water. These pieces were ground into the test tube containing 1 to 2 ml of sterilized distilled water and allowed the bacteria to ooze out from the leaf tissue. One loop with bacterial suspension was streak onto nutrient agar (NA) medium, nutrient agar yeast extract (NYA) and peptone sucrose agar (PSA) medium. The plates were incubated at room temperature (28±2 °C) for 3 to 4 days. Bacterium was identified based on colony character (Arshad *et al.* 2015) and transferred into slant nutrient medium as pure culture. These strains were preserved at 4 °C up to 1 month for further evaluation (Jonit *et al.* 2016).

Source and multiplication of rhizopheric bacteria

Rhizopheric bacterial isolates (*B. subtilis* and *A. xylosoxidans*) were collected from laboratory of Bioactive Compounds, Bio-formulation and Bio-signaling, Plant Pathology Department, Bangladesh Agricultural University, Mymensingh (Sultana *et al.* 2018). *Bacillus subtilis* and *A. xylosoxidans* were sub-cultured on Nutrient Agar and were stored in 20 % glycerol at – 80 ºC for further use.

Preparation of cell culture suspension and cell-free culture filtrates of rhizopheric bacteria

For preparing cell suspension, the purified bacterial cultures were inoculated in Petri dish containing NA media, incubated at 28º C for 24 hours and diluted with sterile distilled water. Cell suspensions were adjusted to 10^8 Colony Forming Unit (CFU/mL) using a spectrophotometer at 600 nm wave length with optical density volume of 0.6 (Azman *et al.* 2017). For cell free culture filtrate preparation, nutrient broth (NB) along with bacterial colony was incubated on a rotary shaker at 30º C for 72 hours (130 rpm) and then centrifuged at 5500 rpm for 25 minutes. The supernatant was collected and filtrated *via* 0.22 µm milipore filter to remove any bacterial cell and stored until later use (Zhu *et al*. 2015).

Screening of *in-vitro* antagonistic activity of rhizopheric bacterial cell culture suspension and cell-free culture filtrates against *X. oryzae* pv. *oryzae*

Dual culture technique was followed to identify the effective strain of rhizopheric bacteria (*B. subtilis* and *A. xylosoxidans*) against *X. oryzae* pv. *oryzae*. Colony of *X. oryzae* pv. *oryzae* was mixed with sterilized Nutrient Agar @ 100 µL/plate. After thorough mixing of *X. oryzae* pv. *oryzae* with NA, the mixture was poured onto sterilized glass petri plates and left for solidification under the laminar air-flow cabinet for 30 mins. After solidification, rhizopheric bacterial culture suspension and cell-free culture filtrate were placed separately at the centre of different petri plates @ 1 µL/plate by using micropipette and incubated at 37º C for 12 hours followed by incubation at 30º C for 24 hours. After 36 hours of incubation, inhibition halos were measured and antimicrobial activity (mm) was expressed as the difference between diameter of inhibition zone and diameter of rhizopheric bacterial colony (Monteiro 2002).

Field Experiment

The *in-vivo* experiment was carried out at the Central Farming System (CFS), Bangladesh Agricultural University, Mymensingh to evaluate the effect of foliar spray of rhizopheric bacterial suspension and their cell-free culture filtrates against *X. oryzae* pv. *oryzae* in natural condition. In total six treatments *viz*. T_0 = Control, T_1 = *Bacillus subtilis* suspension (10^8 CFU/mL), T_2 = *Achromobacter xylosoxidans* suspension (10^8 CFU/mL), T_3 = *Bacillus subtilis* filtrates, T_4 = *Achromobacter xylosoxidans* filtrates, T_5 = Bactroban (40 g/10 L) were sprayed for three times at 15 days interval starting from 30 days after transplanting (DAT) in rice cv. BRRI dhan28. Rice plant naturally infected by *X. oryzae* pv. *oryzae* was considered for data collection. Data on vegetative parameters, disease incidence and severity were recorded at three growth stages *viz.* tillering stage, panicle initiation stage and flowering stage. Harvesting was done after 120 days of transplanting. Data on growth parameters were collected on the following: number of tiller/hill, number of infected tiller/hill, number of leaves/hill, number of infected leaves/hill, number of panicle/hill, number of grain/panicle, panicle length (cm).

Disease assessment

Percent disease incidence was estimated according to the following formula (Rafi *et al.* 2013):

$$\% \text{ Disease Incidence} = \frac{\text{Number of Diseased plants}}{\text{Total Number of plants counted}} \times 100$$

Disease severity index was calculated using an orginal scale from 0 to 9 (IRRI 2004), the disease severity scale was determined according to the formula below.

Percent Disease Severity (PDI) =

$$\frac{\text{Sum of all disease ratings}}{\text{Total No of Number of disease grade} \times \text{Maximum disease grade}} \times 100$$

Experimental designs and analyses

The experiments were laid out in Completely Randomized Design (CRD) for laboratory study and Randomized Complete Block Design (RCBD) for field study with three replications. The collected data were analyzed statistically using MSTAT-C package program. The significance of the difference among the means was calculated by LSD test (Least Significant Difference).

RESULTS AND DISCUSSION

In vitro* antagonistic effect of rhizobacterial cell culture suspensions (*B. subtilis* and *A. xylosoxidans*) on the growth of *X. oryzae* pv. *oryzae

Ten isolates of *B. subtilis* and *A. xylosoxidans* were subjected to dual culture assay to observe the growth suppression activity against *X. oryzae* pv. *oryzae* at 24 hrs intervals (Table 1). At 24 hours after inoculation (HAI) the highest growth was recorded in case of BSL-26(iii) (30.00) followed by *A. xylosoxidans* (29.00), while minimum growth was recorded in BSL-21(6.33). Again, BSL-26(iii) (32.00) followed by *A. xylosoxidans* (31.33) showed highest growth, where minimum growth of *Bacillus* spp. was observed in BSL-21 (7.33) followed by BSL-10, BSL-31 (15.33), BSL-17 (17.33) at 48 HAI. At 72 HAI, the highest growth was observed in BSL-26(iii) (32.00) followed by *A. xylosoxidans (*31.33), where minimum growth of *Bacillus* spp. was recorded in BSL-21 (7.33) followed by BSL-31 (15.33). So, it is revealed that cell suspension of *B. subtilis* [BSL-26(iii)] and *A. xylosoxidans* had the highest growth which render the maximum inhibition ability against *X. oryzae* pv. *oryzae*.

Table 1. Growth supression ability of *Bacillus subtilis* and *Achromobacter xylosoxidans* cell suspensions against *Xanthomonas oryzae* pv. *oryzae* by dual culture assay

Isolates	Growth of bacterial strains at different time interval (mm)		
	24 HPI	48 HPI	72 HPI
BSL-41	21.33 ab	23.00 bc	23.00 bc
BSL-27(ii)	17.00 bc	18.00 c	18.00 c
BSL-8	21.60 ab	29.00 ab	29.66 ab
BSL-17	15.33 bcd	17.33 c	17.33 c
BSL-31	13.00 bcd	15.33cd	15.33 cd
BSL-10	10.67 cd	15.33 cd	17.66 c
BSL-21	6.33 d	7.33 d	7.33 d
BSL-26(iii)	30.00 a	32.00 a	32.00 a
Achromobacter xylosoxidans	29.00 a	31.33 ab	31.33 ab
BSL-11(i)	21.67 ab	27.00 ab	27.00 ab
CV (%)	31.808	23.028	22.544

HPI = Hours Post Inoculation

In vitro* antagonistic effect of rhizobacterial cell-free culture filtrates (*B. subtilis* and *A. xylosoxidans*) on the growth of *X. oryzae* pv. *oryzae

Cell-free culture filtrates of ten isolates of *B. subtilis* and *A. xylosoxidans* were subjected to dual culture assay to observe the growth suppression activity against *X. oryzae* pv. *oryzae* at 24 hrs intervals (Table 2). At 24 HAI BSL-27(ii), BSL-8 (5.33) followed by BSL-41 (5.00) showed the highest growth, where the minimum growth was observed in BSL-17, *A. xylosoxidans* (1.66) followed by BSL-21 (2.00). The highest growth (11.00) of BSL-27(ii) followed by BSL-8(8.33) and BSL-41 (8.00) was recorded at 48 HAI, where the minimum growth was observed in BSL-17 (3.00) followed by BSL-31 (4.00), BSL-10, *A. xylosoxidans* (4.33).

Table 2. Growth supression ability of *Bacillus subtilis* and *Achromobacter xylosoxidans* culture filtrates aganist *Xanthomonas oryzae* pv. *oryzae* by dual culture assay

Isolates	Growth of bacterial strains at different time interval (mm)		
	24 HPI	48 HPI	72 HPI
BSL-41	5.00 a	8.00 abc	8.00 abc
BSL-27(ii)	5.33 a	11.00 a	11.33 a
BSL-8	5.33 a	8.33 ab	8.66 ab
BSL-17	1.66 bc	3.00 de	3.00 de
BSL-31	2.33bc	4.00 d	4.00 d
BSL-10	2.33 bc	4.33 d	4.33 d
BSL-21	2.00 bc	5.33 bcd	5.33 bcd
BSL-26(iii)	4.00 ab	4.66 cd	4.66 cd
Achromobacter xylosoxidans	1.66 bc	4.33 d	4.333 d
BSL-11(i)	2.00 bc	4.00 cd	4.00 cd
CV (%)	48.843	38.974	37.260

HPI = Hours Post Inoculation

At 72 HAI BSL-27(ii) showed the highest (11.33) growth, while the minimum growth was recorded in BSL-17 (3.00) followed by BSL-31(4.00), BSL-10, *A. xylosoxidans* (4.33) indicating that the growth of *X. oryzae* pv. *oryzae* was highly suppressed by cell-free culture filtrates of *B. subtilis* [BSL-26(iii)] and *A. xylosoxidans* compared to untreated control and other isolates. Similar kind of finding was reported by Chen *et al*. (2013) that strains of *B. Subtilis* from natural environments were able to form robust bio-films in defined medium which enhance the growth and activity of bacteria substantially resulting in pathogenic growth suppression. Grover *et al*. (2010) observed that the strain RP24 showed significant *in-vitro* antagonism against a wide range of phyto-pathogenic fungi by producing antibiotics like iturin, surfactin and fengycin. The present research findings hypothesized that bioactive compounds may be present in the native bacterial strains which are responsible for growth suppression of *X. oryzae* pv. *oryzae* as indicated Grover *et al*. (2010) in his study.

Effect of bacterial bio-agents (*B. subtilis* and *A. xylosoxidans*) as foliar application on vegetative-, disease- and reproductive-parameters of rice plants under natural condition in the field

An experiment was conducted in the field to examine the efficacy of cell suspensions and cell free culture filtrates of *B. subtilis* [BSL-26(iii)] and *A. xylosoxidans* on the vegetative growth promotion, disease suppression and influence on reproductive parameters of rice cv. BRRI dhan28 at different growth stages. Vegetative growth parameters *viz.* number of tiller/hill, number of leaves/hill and reproductive parameters *viz.* number of panicle/hill, number of grain/panicle, panicle length (cm) were significantly influenced by foliar application of rhizo-bacterial cell suspensions and cell free culture filtrates compared to control treatment at different growth stages (Table 3, 4, 5 and 6).

At 30 DAT, tillers/hill and number of leaves/hill were significantly increased by the application of all treatments. But, statistically similar and highest number of tillers and leaves/hill were recorded in T_2 (*Achromobacter xylosoxidans* suspension @ 10^8 CFU/mL) (9.67, 27.67), T_4 (*Achromobacter xylosoxidans* filtrate) (9.00, 26.33) and T_1 (*Bacillus subtilis* suspension @ 10^8 CFU/mL) (8.33, 26.33) compared to untreated control (7.67, 23.00). Moreover, infected tillers (5.33) and leaves/hill (8.00) were recorded highest in T_2 (*Achromobacter xylosoxidans* suspension@ 10^8 CFU/mL) and T_0 (Control) respectively. On the contrary, significant reductions in number of infected tiller (3.33) and leaves/ hill (4.00) were recorded in T_1 (*Bacillus subtilis* suspension @ 10^8 CFU/mL) and T_3 (*Bacillus subtilis* filtrate).

At 60 DAT, the highest number of tillers (17.67) and leaves/hill (64.00) were recorded in both T_4 (*Achromobacter xylosoxidans* filtrate) and T_1 (*Bacillus subtilis* suspension @ 10^8 CFU/mL), while the rest of the treatments showed similar effect. On the contrary, significant and lowest numbers of infected tillers and leaves/ hill were recorded in all treatments compared to untreated control.

At 90 DAT, number of tillers (17.67) and leaves/hill (64.00) were recorded highest in T_4 (*Achromobacter xylosoxidans* filtrate) and T_1 (*Bacillus subtilis* suspension @ 10^8 CFU/mL), while rest of the treatments showed similar effect. On the other hand, least numbers of infected tillers (9.33) and leaves/ hill (20.00) were recorded in T_1 where cell suspension of

Bacillus subtilis @ 10^8 CFU/mL was applied compared to T_0 (Control). Statistically similar and the lowest numbers of infected tillers and leaves/ hill were also recorded in T_2 (*Achromobacter xylosoxidans* suspension @ 10^8 CFU/mL), T_3 (*Bacillus subtilis* filtrate) and T_4 (*Achromobacter xylosoxidans* filtrate).

Table 3. Effect of different treatments on vegetative growth of BRRI dhan28 in the field under natural condition at 30 DAT (tillering stage)

Treatments	No. of tiller/hill	No. of infected tiller/hill	No. of leaves/hill	No. of infected leaves/hill
T_0	7.67 cd	5.00 b	23.00 b	8.00 a
T_1	8.33 bc	3.33 d	26.33 a	4.00 d
T_2	9.67 a	5.33a	27.67 a	5.00 cd
T_3	6.33 e	3.30 d	19.00 c	4.00 d
T_4	9.00 ab	4.00 c	26.33 a	7.00 ab
T_5	7.33 d	2.67 e	22.00 b	6.00 bc
Level of significance	0.01	0.01	0.01	0.01
CV (%)	5.29	4.63	4.41	9.39

Here, Column having the similar letter did not differ significantly at 0.05 level of significance
[T_0 = Control, T_1 = *Bacillus subtilis* suspension (10^8 CFU/mL), T_2 = *Achromobacter xylosoxidans* suspension (10^8 CFU/mL), T_3 = *Bacillus subtilis* filtrate, T_4 = *Achromobacter xylosoxidans* filtrate, T_5 = Bactroban (40 g/10 L), CV = Co-efficient of variation]

All the treatments showed highest (15.67) and statistically similar effect on total number of panicle/hill compared to untreated control treatment T_0 (14.00) at 120 DAT. Again, significantly highest (139.30) number of grains/panicle was recorded in T_1 (*Bacillus subtilis* suspension @ 10^8 CFU/mL) followed by T_5 (Bactroban @ 40 g/10 L) (137.70), while the lowest number of grains/panicle was observed in untreated control (T_0). However, significant increment in panicle length was recorded in all the treatments except T_0 where no treatment was applied.

Table 4. Effect of different treatments on vegetative growth of BRRI dhan28 in the field under natural condition at 60 DAT (panicle initiation stage)

Treatments	No. of tiller/hill	No. of infected tiller/hill	No. of leaves/hill	No. of infected leaves/hill
T_0	15.00 b	9.67 a	55.00 b	26.00a
T_1	15.33 b	6.33 c	64.00 a	11.33 c
T_2	14.67 b	6.33 c	53.33 b	12.67 b
T_3	14.67 b	6.33 c	56.00 b	13.00 b
T_4	17.67 a	6.00 c	64.00 a	13.33 b
T_5	13.67 b	7.00 b	54.67 b	13.00 b
Level of significance	0.01	0.01	0.01	0.01
CV (%)	5.72	3.72	3.24	4.66

Here, Column having the similar letter do not differ significantly at 0.05 level of significance
[T_0 = Control, T_1 = *Bacillus subtilis* suspension (10^8 CFU/mL), T_2 = *Achromobacter xylosoxidans* suspension (10^8 CFU/mL), T_3 = *Bacillus subtilis* filtrate, T_4 = *Achromobacter xylosoxidans* filtrate, T_5 = Bactroban (40 g/10 L), CV = Co-efficient of variation]

Table 5. Effect of different treatments on vegetative growth of BRRI dhan28 in the field under natural condition at 90 DAT (flowering stage)

Treatments	No. of tiller/hill	No. of infected tiller/hill	No. of leaves/hill	No. of infected leaves/hill
T_0	15.00 b	12.00 a	55.00 b	29.67 a
T_1	15.00 b	9.33 c	64.00 a	20.00 d
T_2	14.67 b	10.67 b	53.33 b	18.00 d
T_3	14.67 b	9.33 c	56.00 b	26.33 b
T_4	17.67 a	9.67c	64.00 a	25.33 b
T_5	13.67 b	10.00 bc	54.67 b	22.67 c
Level of significance	0.01	0.01	0.01	0.01
CV (%)	4.67	4.50	3.61	4.52

Here, Column having the similar letter do not differ significantly at 0.05 level of significance
[T_0 = Control, T_1 = *Bacillus subtilis* suspension (10^8 CFU/mL), T_2 = *Achromobacter xylosoxidans* suspension (10^8 CFU/mL), T_3 = *Bacillus subtilis* filtrate, T_4 = *Achromobacter xylosoxidans* filtrate, T_5 = Bactroban (40 g/10 L), CV = Co-efficient of variation]

Table 6. Effect of different treatments on the reproductive growth of BRRI dhan28 in the field under natural condition at 120 DAT (harvesting stage)

Treatments	Total panicle/ hill	No. of grain/ panicle	Length of panicle (cm)
T_0	14.00 b	115.70 d	18.00 d
T_1	15.67 a	139.30 a	22.33 abc
T_2	15.67 a	121.70 c	23.00 a
T_3	15.67 a	123.00 c	22.67 ab
T_4	15.67 a	132.30 b	21.67 c
T_5	15.33 a	137.70 a	22.00 bc
Level of significance	0.01	0.01	0.01
CV (%)	3.72	2.28	1.91

Here, Column having the similar letter do not differ significantly at 0.05 level of significance
[T_0 = Control, T_1 = *Bacillus subtilis* suspension (10^8 CFU/mL), T_2 = *Achromobacter xylosoxidans* suspension (10^8 CFU/mL), T_3 = *Bacillus subtilis* filtrate, T_4 = *Achromobacter xylosoxidans* filtrate, T_5 = Bactroban (40 g/10 L), CV = Co-efficient of variation]

Most of the treatments in this experiment, significantly increased vegetative and reproductive parameters and reduced disease parameters by both cell culture suspension and cell-free culture filtrates; but, application of cell suspension of *Bacillus subtilis* @ 10^8 CFU/mL on the foliage showed the superior performances at all stages compared to untreated control. The findings are in similar line where growth promotion was also observed by the application of plant growth promoting rhizobacteria (Pierson and Weller 1994, Duffy and Weller 1995). The efficiency of cell suspension of rhizobacteria is primarily concerned with their ability to produce auxin, solubilization of phosphorus and activity ACC deaminase; however, other factors such as its ability to inhibit pathogens, which can indirectly influence the growth of plants (Jalili *et al.* 2009, Roca *et al.* 2013). Moreover, cell suspension of rhizopheric bacteria has been applied to various crops to enhance growth, seedling emergence and crop reproductive parameters (Herman *et al*. 2008, Nayaka *et al*. 2009, Choong-Min *et al.* 2007, Saravanakumar *et al.* 2007). Rhizobial inoculants have also been reported to improve nutrient uptake, growth, seedling vigor and yield of rice (Biswas *et al.* 2000).

Effect of bacterial bio-agents (*B. subtilis* and *A. xylosoxidans*) on the incidence and severity of BLB of rice under natural condition in the field

The disease incidence of BLB of rice was significantly influenced by the foliar spray of bacterial cell culture suspensions and cell-free culture filtrates at 30, 60 and 90 DAT (Figure 1). At 30 DAT, the lowest BLB incidence (19.00 %) was recorded in Bactroban (40 g/10 L). Significantly lower incidence was also recorded by foliar application of *Bacillus subtilis* suspension @ 10^8 CFU/mL (26.00 %) and *Bacillus subtilis* filtrates (30.00 %) compared to untreated control. At both 60 and 90 DAT, BLB incidence of rice was recorded lowest by Bactroban (40 g/10 L). Foliar application of *Bacillus subtilis* suspension (10^8 CFU/mL) also resulted significant reduction in incidence of BLB of rice at 60 DAT (37.00 %) and 90 DAT (48.00 %) compared to respective untreated control.

Severity of BLB of rice was also significantly influenced by different treatments at different growth stages (Figure 2). At 30 DAT, the lowest severity of BLB (17.00 %) was found in Bactroban (40 g/10 L), while the lower severity was also recorded by *Bacillus subtilis* suspension @ 10^8 CFU/mL (21.00 %) and *Bacillus subtilis* filtrates (22.00 %) compared to untreated control (32.00 %). At 60 and 90 DAT, the lowest BLB severity of rice was recorded by Bactroban (40 g/10 L). Significantly lower BLB severity was also recorded by *Bacillus subtilis* suspension (10^8 CFU/mL) (26.00 %) at 60 DAT and *Bacillus subtilis* suspension (10^8 CFU/mL) (36.00 %) at 90 DAT compared to untreated control. Mardanova *et al*. (2016) reported that *B. subtilis* strain GM2 promoted growth of wheat but only GM5 strain was able to protect wheat seedlings from *Fusarium oxysporum* infection by producing a number of hydrolytic enzymes as well as antimicrobial metabolites (ammonia and HCN) which is in the similar line with our present study. Udayashankar *et al.* (2011) found that strains of *B. subtilis* (GBO3) and *B. Pumilus* (SE34) showed higher levels of disease suppression of BLB (58 % and 71 %) when applied as fresh cell suspension.

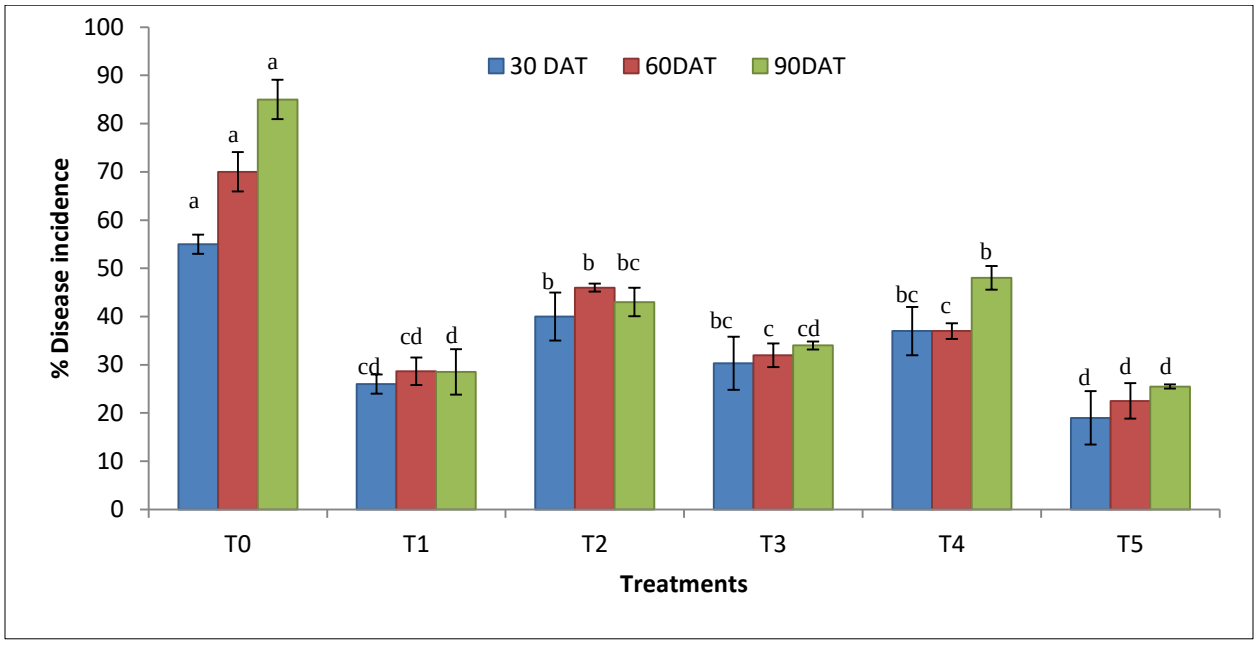

Figure 1. Effect of different treatments on percent disease incidence of BLB of rice BRRI dhan28

T_0 = Control, T_1 = *Bacillus subtilis* suspension (10^8 CFU/mL), T_2 = *Achromobacter xylosoxidans* suspension (10^8 CFU/mL), T_3 = *Bacillus subtilis* filtrates, T_4 = *Achromobacter xylosoxidans* filtrates and T_5 = Bactroban (40 g/10L); Colum having the similar letter do not differ significantly at 0.05 level of significance

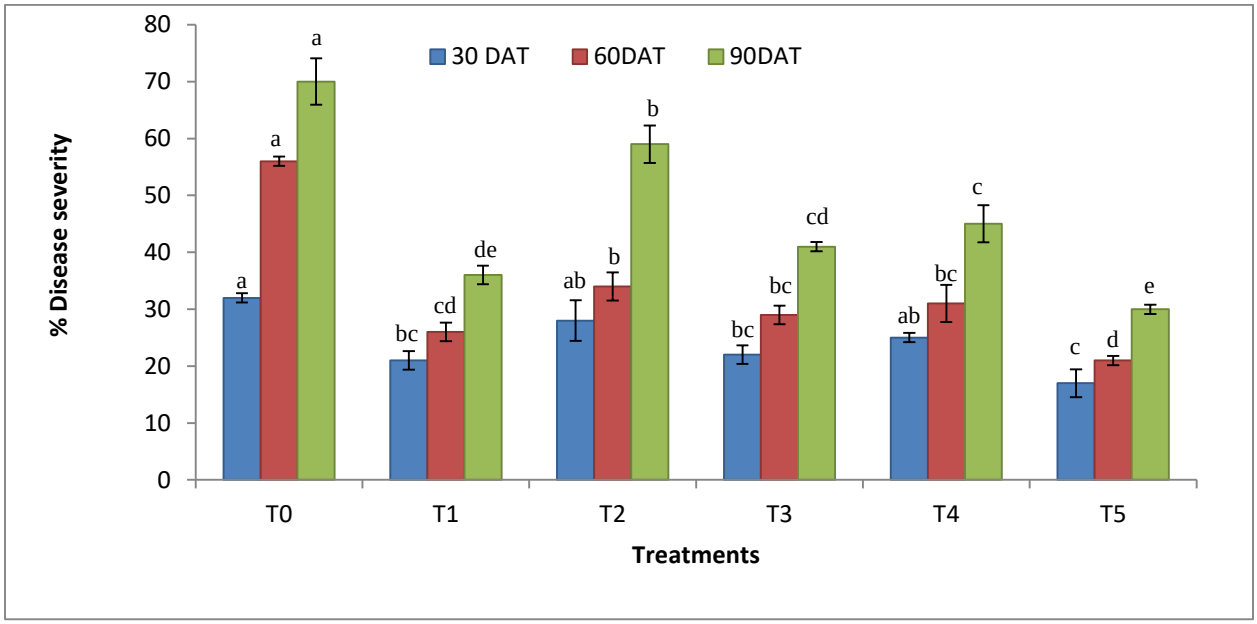

Figure 2. Effect of different treatments on percent disease severity of BLB of rice BRRI dhan28

T_0 = Control, T_1 = *Bacillus subtilis* suspension (10^8 CFU/mL), T_2 = *Achromobacter xylosoxidans* suspension (10^8 CFU/mL), T_3 = *Bacillus subtilis* filtrates, T_4 = *Achromobacter xylosoxidans* filtrates and T_5 = Bactroban (40 g/10L); Colum having the similar letter do not differ significantly at 0.05 level of significance

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