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IN VITRO EVALUATION OF BACTERIAL ENDOPHYTES AGAINST MAJOR SEEDBORNE PATHOGENS OF RICE GRAINS

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ABSTRACT

The present study was conducted with an aim to find out the efficacy of bacterial endophytes against major seedborne pathogens of rice grains. Twenty three seedborne pathogens were isolated by Agar plate method from 26 rice varieties collected from Assam and Meghalaya. Among 23 seed borne pathogens, *Curvularia lunata*, *Fusarium moniliforme*, *Aspergillus cumulatus*, *Phoma sorghina*, *Aspergillus glaucus Xanthomonas oryzae* pv. *oryzae* and *Bipolaris oryzae* were predominant and frequently isolated. Out of 23 endophytes (15 *Bacillus* spp. and 8 *Pseudomonas* spp.) isolated from rice leaves, five *Bacillus* spp. were more effective against growth of fungal pathogens, whereas four *Bacillus* spp. showed effectiveness against *X. oryzae* pv. *oryzae*.

Keywords: Endophytes, Rice, Seedborne pathogens.

More than 3.5 billion of the world population depends on rice for 20% or more of the daily calorie intake (Sanchez *et al*., 2020). But rice crop is severely affected by different diseases which results in considerable yield loss. Annual losses due to rice diseases are estimated about 10-15% worldwide (Kumar *et al*., 2013). Some diseases *viz*., blast (*Pyricularia grisea*), brown spot (*Bipolaris oryzae*), sheath rot (*Sarocladium oryzae*) and grain discoloration (fungal complex) are economically important due to their seedborne nature (Chhabra and Vij, 2020). Rice is attacked by more than 70 diseases which are caused by fungi, bacteria, viruses and nematodes (Mandipa *et al*. 2013). However, majority of the diseases are fungal diseases. Fungi are known to cause 42 per cent yield loss under favourable conditions (Archana and Prakash, 2013). In present day scenario, where everyone is shifting towards organic cultivation due to the harmful effects of the chemical treatment, use of endophytes plays an important role in the management of different diseases of rice crop. Bacterial endophytes suppress plant pathogens following mechanisms similar to that of rhizospheric bacteria. Basha and Ulaganathan (2002) found high antagonistic activity against *C. lunata* by *Bacillus* sp. strain BC121 under *in vitro* condition in suppressing the mycelial growth of the pathogen up to 60%. Naik *et al.* (2016) found that among the 23 endophytic *Bacillus* isolates, strain BA_2 provided maximum inhibition (52.22%) of *B. oryzae.* There are different modes of action of bio-control bacteria, including inhibition of the pathogens by antimicrobial compounds, competition for iron through siderophores production, parasitism involving the production of extracellular enzymes including chitinases and proteases that destroy the cell walls of phytopathogenic fungi and induce resistance mechanisms in plants (Shyamala and Sivakumaar, 2012).

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MATERIALS AND METHODS

Isolation of the seedborne pathogens

The present study was carried out in the Department of Plant Pathology Laboratory, School of Crop Protection, College of Post Graduate Studies in Agricultural Sciences, (Central Agricultural University), Imphal, Umiam, Meghalaya in the year 2019-20. Twenty three seedborne pathogens were isolated by Agar plate method (ISTA, 2013) from 26 rice varieties collected from Assam and Meghalaya. Among 23 seed borne pathogens, *Curvularia lunata*, *Fusarium moniliforme*, *Aspergillus cumulatus, Phoma sorghina*, *Aspergillus glaucus, Xanthomonas oryzae* pv. *oryzae* and *Bipolaris oryzae* were predominant and frequently isolated.

Identification of bacterial and fungal pathogens

Identification of bacterial and fungal pathogens was done by observing the morphological characteristics as described Danquash *et al*. (1976), Suleiman and Hussain (1984), Suresh *et al.* (2013) and Samanta *et al.* (2014).

Isolation of bacterial endophytes

Isolation was done by following the method of Zinniel *et al*. (2002) with slight modifications. Healthy and undamaged rice leaves (from 45th day old plant) collected from experimental field were washed in

distilled water, surface sterilized by rinsing with 1% sodium hypochlorite for 1 min followed by immersing in 70% ethanol for 1-2 min and rinsing again in sterile distilled water 3 times. The samples were cut into 2-3 cm size small pieces and macerated using phosphate saline buffer (pH 7.2) with a sterilized mortar and pestle. The extract solution was then kept in the incubator for 3 h at 28±1°C. For isolation of Bacillus spp., 1.5 ml of the aliquot was treated in hot water bath at 60-80°C for 5 min to eliminate other unwanted microorganisms. This was additional step followed specifically for Bacillus. This was followed by serial dilution up to 10^{-5}. 0.1 ml from this final aliquot was plated in Nutrient Agar (NA) medium by Pour Plate method. For isolation of Pseudomonads, both NA and King's B (KMB) media were used.

Detection and purification of *Bacillus* and *Pseudomonas* isolates

The bacterial colonies developed in the dilution plates of NA and KMB agar were observed under U.V. transilluminator for the presence of greenish yellow fluorescent pigment and only such representative fluorescent colonies were selected.

The single colonies were then picked up and streaked in fresh NA medium and KMB media respectively with the help of a sterile loop to obtain pure culture. They were incubated at 28±1°C for 24 h. The colony morphologies of all the isolates

Evaluation of antagonistic effects of bacterial endophytes against major seed borne pathogens of rice grains

Dual culture assay against *Curvularia lunata*

Among 22 fungal pathogens isolated from husk and kernel of different rice varieties, 6 were selected and evaluated for their interaction with the beneficial endophytes. Among these 6 pathogens, *Curvularia lunata* occurred dominantly. All the 23 bacterial isolates were first screened against *C. lunata* under *in vitro* by Dual culture assay (Ganesan and Gnanamanickam, 1987). All the treatments were replicated 3 times. The plates were incubated at 28 ± 1°C till the control plates attained full growth. Inhibition percentage was calculated (Vincent, 1947) as under:

$$\text{Inhibition (\%)} = \frac{C-T}{T} \times 100$$

Where, C = radial growth of pathogen in control (cm)

T = radial growth of pathogen in treatment (cm)

Dual culture assay against other major seed borne pathogens of rice grains

Out of 23 bacterial endophytes evaluated against *C. lunata*, 5 best showing maximum inhibition were selected and evaluated for their antagonistic potential against remaining 5 major fungal pathogens namely i) *Aspergillus cumulatus* ii) *Aspergillus glaucus* iii) *Bipolaris oryzae* iv) *Fusarium moniliforme* v) *Phoma sorghina in vitro* by Dual culture assay.

In vitro evaluation of bacterial endophytes against bacterial seed borne pathogen of rice grain

The efficacy of 5 best bacterial antagonists used against 5 fungal seedborne pathogens was tested against the growth of *Xanthomonas oryzae* pv. *oryzae* (Xoo) *in vitro* by standard paper disc method of Thornberry (1950). The efficacy of the different bacterial antagonists was assessed by measuring the zone of inhibition surrounding the filter paper disc after the incubation period. Comparison was made with the control plates. Inhibition percentage was calculated using the formula described by Vincent (1947).

$$\text{Inhibition (\%)} = \frac{C-T}{T} \times 100$$

Where, C = growth of bacteria in control (cm)

T = growth of bacteria in treatment (cm)

RESULTS AND DISCUSSION

Isolation of bacterial endophytes

A total of twenty three bacterial endophyte isolates were obtained from different cultivars of healthy rice leaves. Among 23 isolates, 15 isolates were *Bacillus* spp. and 8 isolates were *Pseudomonas* spp. The results are in accordance with the findings of Elbeltagy *et al*. (2000) who isolated bacterial endophytes both from wild and cultivated rice varieties. Similarly, Sev *et al*. (2016) isolated a total of 18 morphologically dissimilar bacterial endophytes from different varieties of rice.

Evaluation of antagonistic effects of bacterial endophytes against major seed borne pathogens of rice grains

Dual culture assay against Curvularia lunata

Twenty three bacterial endophytes were evaluated against *C. lunata*. Only 9 isolates showed 50% and above inhibition of mycelial growth of the pathogen (Table1 and Fig. 1). Among these 9 isolates, only 5 isolates of *Bacillus* were found effective against the pathogen. Isolate BC 15 showed inhibition of 59.79%

Table 1. Dual culture assay against *C. lunata*

Isolate	Growth (cm)	Inhibition (%)
BC 1	4.21±0.07 (2.05)	51.73±0.86^{bcd} (7.19)
BC 2	6.04±0.09 (2.45)	31.76±0.15^{ghi} (5.63)
BC 3	3.54±0.062 (1.88)	59.44±1.26^{a} (7.71)
BC 4	4.75±0.15 (2.18)	48.33±0.23^{def} (6.95)
BC 5	5.82±0.09 (2.41)	33.25±1.07^{gh} (5.76)
BC 6	3.85±0.10 (1.96)	57.04±1.15^{ab} (7.55)
BC 7	5.57±0.21 (2.36)	36.34±2.51^{gh} (6.02)
BC 8	4.02±0.07 (2.006)	55.01±0.86^{abc} (7.41)
BC 9	4.88±0.13 (2.21)	44.02±1.57^{f} (6.63)
BC 10	4.22±0.16 (2.05)	52.80±1.86^{bcd} (7.26)
BC 11	4.94±0.08 (2.22)	43.37±0.98^{f} (6.58)
BC 12	7.01±0.23 (2.64)	19.62±2.67^{j} (4.42)
BC 13	4.76±0.28 (2.18)	45.39±3.26^{ef} (6.73)
BC 14	7.34±0.14 (2.71)	15.84±1.70^{f} (3.97)
BC 15	3.64±0.08 (1.90)	59.79±1.30^{a} (7.73)
FP 1	4.25±0.12 (2.06)	51.31±1.46^{cd} (7.16)
FP 2	4.32±0.02 (2.07)	50.47±0.33^{cde} (7.10)
FP 3	4.31±0.07 (2.07)	50.01±0.86^{bcd} (7.07)
FP 4	5.98±0.13 (2.44)	31.46±1.53^{hi} (5.60)
FP 5	5.44±0.13 (2.33)	37.11±0.22^{g} (6.09)
FP 6	7.32±0.20 (2.70)	16.11±2.31^{j} (4.01)
FP 7	6.40±0.29 (2.53)	26.61±3.36^{i} (5.15)
FP 8	7.24±0.41 (2.69)	16.99±4.79^{j} (4.12)
Control	9.00±0.00 (3.00)	0.00±0.00^{k} (0.70)
CD (0.05)	0.70	5.51
SEm (±)	0.17	1.98

Data in the table are mean values of 3 replicates ; Values in parentheses are square root transformed; Means in the same column followed by different letters are significantly different at p = 0.05

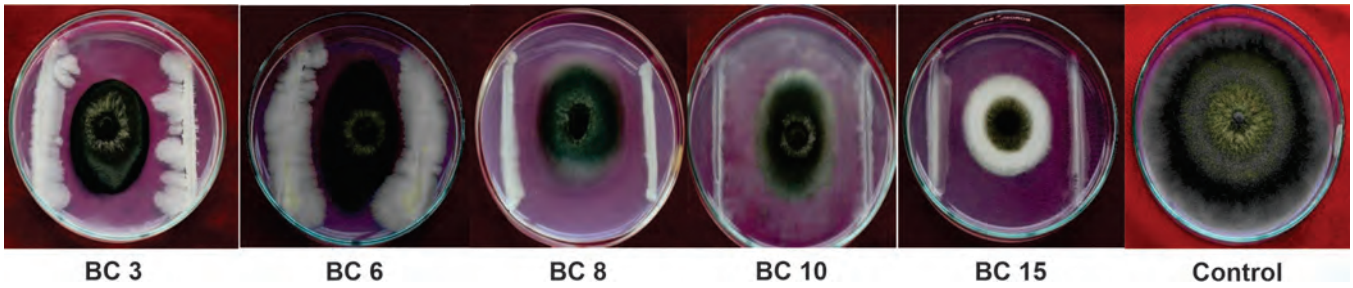

Fig. 1. Dual culture assay against *C. lunata*

followed by isolates BC 3, BC 6, BC 8 and BC 10 with inhibition of 59.44, 57.04, 55.01 and 52.80% respectively and were found to be statistically at par with each other. These best 5 isolates were further evaluated against other major seed borne pathogens of rice grains. Similar findings were reported by Basha and Ulaganathan (2002) who found high antagonistic activity against *C. lunata* by *Bacillus* sp. strain BC121 under *in vitro* condition in suppressing the mycelial growth of the pathogen upto 60%. Similarly, Sumangala *et al*. (2008) tested efficacy *of Bacillus subtilis* against *C. lunata in vitro* and found most effective (97.77%) in inhibiting the mycelial growth of the pathogen.

Dual culture assay against other major seed borne fungal pathogens of rice grains

Dual culture assay against Aspergillus cumulatus

Out of 5 isolates of *Bacillus*, 4 isolates showed more than 50% inhibition of mycelial growth of *A. cumulatus*. Isolate BC 6 showed highest inhibition of 61.21% followed by isolates BC 8, BC 15, BC 3, BC 10 with inhibition of 58.51, 57.62, 56.00 and 49.12% respectively and were found to be statistically at par with each other (Table 2 and Fig. 2). The present findings corroborate the findings of Gong *et al*. (2014)

Table 2. Dual culture assay against *A. cumulatus*

Isolate	Growth (cm)	Inhibition (%)
BC 3	3.89±0.09 (1.97)	56.00±1.13[b] (7.48)
BC 6	3.44±0.10 (1.85)	61.21±1.20[a] (7.82)
BC 8	3.68±0.03 (1.91)	58.51±0.37[ab] (7.64)
BC 10	4.57±0.10 (2.08)	49.12±1.19[c] (7.19)
BC 15	3.757±0.067 (1.938)	57.62±0.77[b] (7.59)
Control	9.000±0.000 (3.00)	0.00±0.00[d] (0.70)
CD (0.05)	0.52	4.38
SEm (±)	0.17	1.39

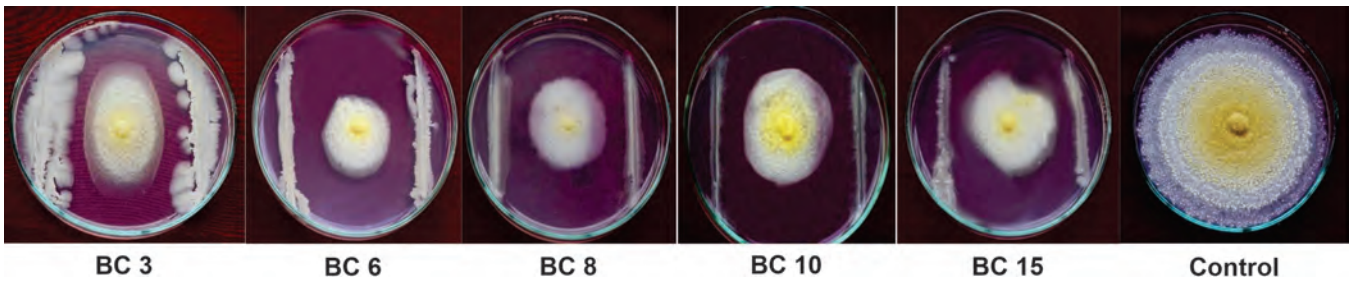

Fig. 2. Dual culture assay against *A. cumulatus*

Table 3. Dual culture assay against *A. glaucus*

Isolates	Growth (cm)	Inhibition (%)
BC 3	3.87±0.39 (1.96)	54.67±3.69[b] (7.39)
BC 6	3.71±0.11 (1.92)	55.67±1.35[b] (7.46)
BC 8	2.33±0.02 (1.52)	72.16±0.27[a] (8.49)
BC 10	3.38±0.09 (1.84)	59.53±1.16[b] (7.71)
BC 15	3.54±0.12 (1.88)	57.64±1.64[b] (7.59)
Control	9.00±0.00 (3.00)	0.00±0.00[c] (0.70)
CD (0.05)	0.52	6.25
SEm (±)	0.16	2.00

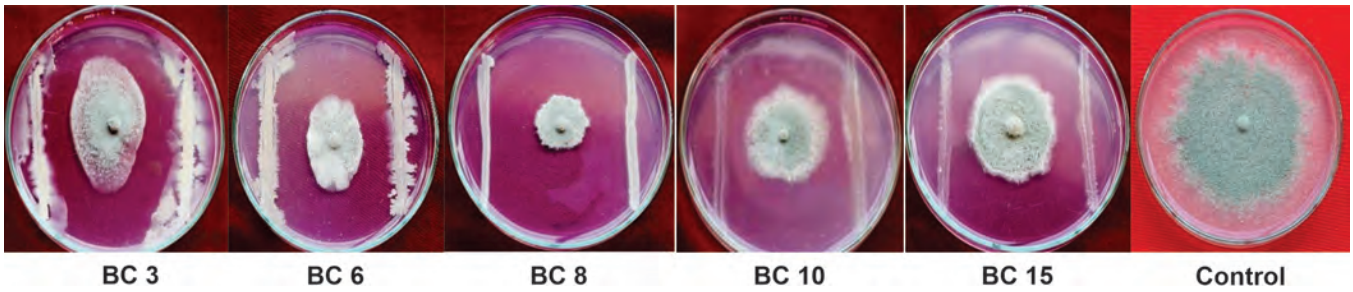

Fig. 3. Dual culture assay against *A. glaucus*

who reported that *B. subtilis* strain fmbJ could suppress the growth of *A. flavus* with an inhibition rate of 85.72% under *in vitro* conditions by the production of antifungal substance bacilomycin D.

Dual culture assay against Aspergillus glaucus

The 5 isolates of *Bacillus* were very successful in inhibiting the mycelial growth of *A. glaucus*. Isolate BC 8 showed highest inhibition of 72.16% followed by isolates BC 10, BC 15, BC 6, BC 3 with inhibition of 59.53, 57.64, 55.67 and 54.67% respectively and were found to be statistically at par with each other (Table 3 and Fig. 3). Podile and Prakash (1996) also showed the successful inhibition of *A. niger* by *B. subtilis* strain AF 1 by suppressing 90% of fungal growth. Similarly, Zieden (2006) reported that 3 isolates of *B. subtilis* suppressed the growth of *A. niger* under field conditions and increased the pod yield.

Dual culture assay against Bipolaris oryzae

Out of 5 isolates of *Bacillus*, 4 isolates showed more than 50% inhibition of mycelial growth of *B. oryzae*. Isolate BC 6 showed highest inhibition of 56.49% followed by isolates BC 3, BC 8, BC 10, BC 15 with inhibition of 56.03, 54.80, 52.37 and 48.15% respectively and were found to be statistically at par with each other (Table 4 and Fig. 4). Similar findings were reported by Kumawat *et al*. (2010) who found the maximum inhibition of *B. oryzae* by *Trichoderma harzianum* (73.335) followed by *B. subtilis* and *P. fluorescens*. Similarly, Naik *et al.* (2016) found that among the 23 endophytic *Bacillus* isolates, strain BA_2 provided maximum inhibition of 52.22% over control.

Dual culture assay against Fusarium moniliforme

The 5 isolates of *Bacillus* showed good result in inhibiting the mycelial growth of *F. moniliforme*. Isolate BC 8 showed highest inhibition of 61.18% followed by isolates BC 15, BC 6, BC 10, BC 3 with inhibition of 60.81, 60.77, 57.60 and, 51.84% respectively and were found to be statistically at par with each other (Table 5 and Fig. 5). The present findings agree with the results of Pal *et al*. (2001) who reported strong antagonism against *F. moniliforme* by isolate *Bacillus* sp. MR-11(2) under *in vitro* condition as it produced an inhibition zone of 15 mm. Similarly, Kazempour and Elahinia (2007) who reported maximum inhibition of *F. moniliforme* by *P. fluorescens* isolate F15 (55%) followed by *B. cereus* isolate F35 (48%) under *in vitro* condition.

Table 4. Dual culture assay against *B. oryzae*

Isolates	Growth (cm)	Inhibition (%)
BC 3	4.10±0.10 (2.02)	54.03±1.18[a] (7.38)
BC 6	3.91±0.21 (1.97)	56.49±2.33[a] (7.51)
BC 8	4.06±0.06 (2.01)	54.80±0.72[b] (7.40)
BC 10	4.37±0.02 (2.09)	51.37±0.32[bc] (7.16)
BC 15	4.57±0.12 (2.13)	48.15±0.25[c] (7.00)
Control	9.00±0.00 (3.00)	0.00±0.00[d] (0.70)
CD (0.05)	0.50	4.94
SEm **(±)**	0.16	1.85

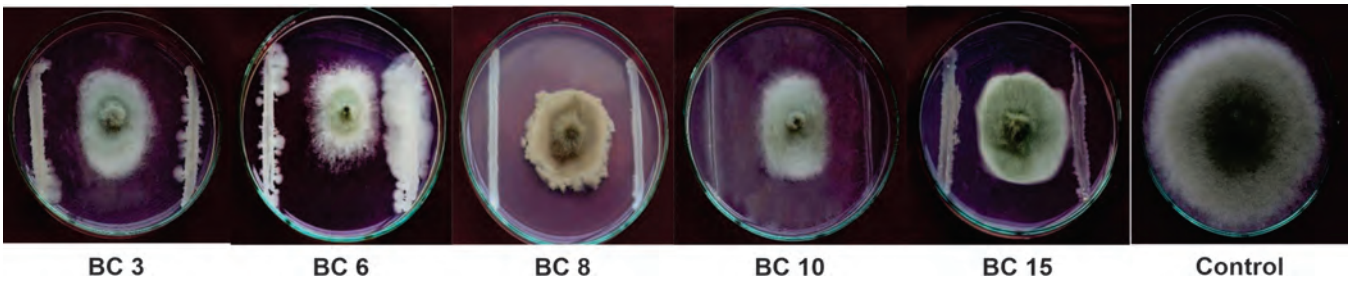

Fig. 3. Dual culture assay against *B. oryzae*

Table 5. Dual culture assay against *F. moniliforme*

Isolates	Growth (cm)	Inhibition (%)
BC 3	4.33±0.02 (2.08)	51.84±0.22[b] (7.20)
BC 6	3.56±0.10 (1.88)	60.77±1.20[a] (7.79)
BC 8	3.53±0.08 (1.87)	61.18±0.88[a] (7.82)
BC 10	3.84±0.01 (1.96)	57.60±0.15[b] (7.58)
BC15	3.56±0.03 (1.88)	60.81±0.396[a] (7.798)
Control	9.00±0.00 (3.00)	0.00±0.00[c] (0.70)
CD (0.05)	0.32	2.16
SEm (±)	0.10	0.69

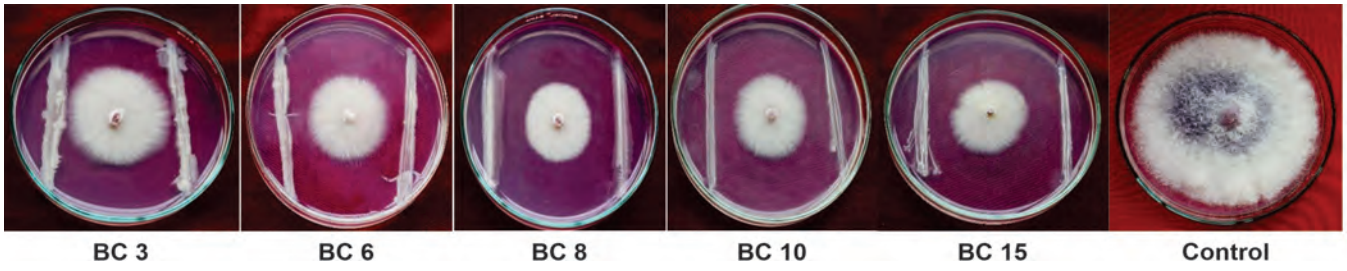

Fig. 5. Dual culture assay against *F. moniliforme*

Table 6. Dual culture assay against *P. sorghina*

Isolates	Growth (cm)	Inhibition (%)
BC 3	3.87±0.28 (1.96)	56.97±3.28[b] (7.54)
BC 6	3.95±0.13 (1.98)	56.06±1.47[b] (7.48)
BC 8	3.31±0.02 (1.81)	63.39±0.23[a] (7.96)
BC 10	3.76±0.02 (1.93)	58.20±0.22[ab] (7.61)
BC 15	3.41±0.44 (1.84)	62.25±0.49[a] (7.89)
Control	9.00±0.00 (3.00)	0.00±0.00[c] (0.70)
CD (0.05)	0.45	5.13
SEm (±)	0.13	1.39

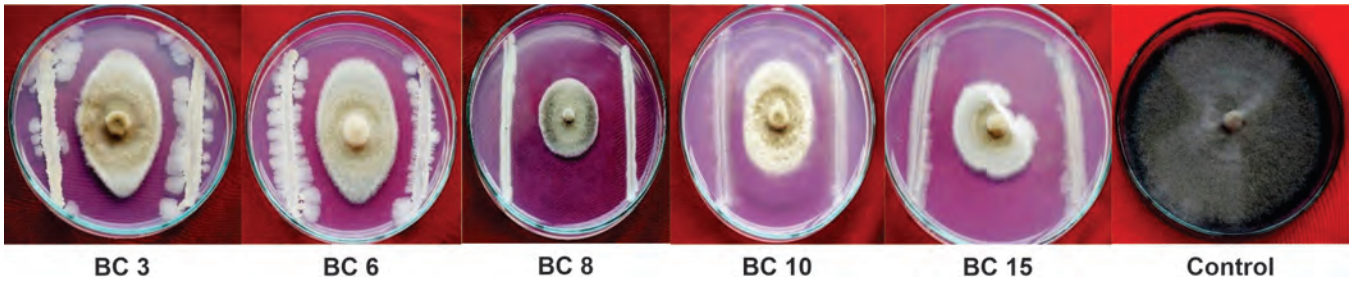

Fig. 6. Dual culture assay against *P. sorghina*

Dual culture assay against *Phoma sorghina*

The 5 isolates of *Bacillus* showed promising result in inhibiting the mycelial growth of *P. sorghina*. Isolate BC 8 showed highest inhibition of 63.39% followed by isolates BC 15, BC 10, BC 3, BC 6 with inhibition of 62.25, 58.20, 56.97 and 56.06% respectively and were found to be statistically at par with each other (Table 6 and Fig. 6). Trojanowska *et al*. (1998) also stated that 9 strains of *Bacillus* sp. showed some activity against *Phoma* sp.

In vitro evaluation of bacterial endophytes against Xoo

Out of 5 isolates of *Bacillus*, 4 isolates showed zone of inhibition of the pathogen. Isolate BC 3 showed highest inhibition of 1.46 cm followed by isolates BC 6, BC 10, BC 8 with inhibition of 1.27, 1.07 and 0.87 cm, respectively and were found to be statistically at par with each other (Table 7 and Fig. 7). Isolate BC 15 failed to produce inhibition of the pathogen. Similar findings were reported by Chen *et al*. (1997) who reported the antagonism activity of *B. subtilis* strain A 30 against Xoo with an inhibition of 58.10% under *in vitro* conditions. Other similar antagonism activities were reported by Xu *et al*. (1998) and Tong *et al*. (1999) by the *B. subtilis* strains B826 and B034 respectively.

Table 7. *In vitro* evaluation of bacterial endophytes against Xoo

Isolate	Inhibition zone (cm)
BC 3	1.46±0.29[a] (1.20)
BC 6	1.27±0.11[b] (1.12)
BC 8	0.87±0.03[d] (0.93)
BC 10	1.07±0.020[c] (1.03)
BC 15	0.00±0.00[e] (0.70)
Control	0.00±0.00[e] (0.70)
CD (0.05)	0.45
SEm (±)	0.11

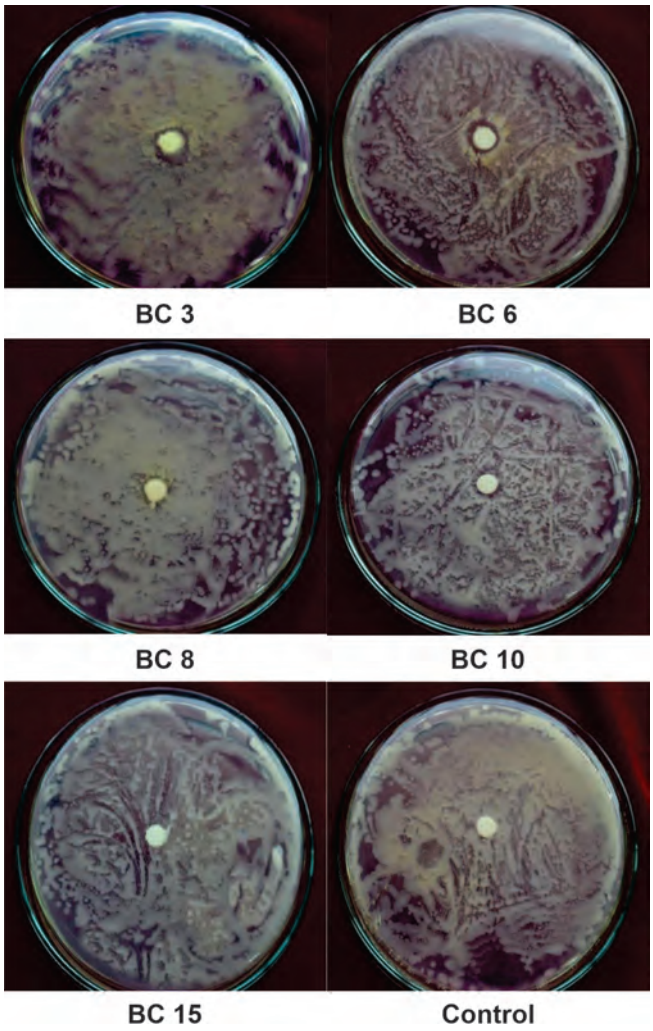

Fig. 7. Inhibition zone by isolates of *Bacillus* against Xoo

Authors' contribution

Conceptualization of research work and designing of experiments (RKT.); Execution of field/lab experiments and data collection (JD); Analysis of data and interpretation (JD, RKT); Preparation of manuscript (JD)

LITERATURE CITED

Archana B P and Prakash H S 2013. Survey of seed-borne fungi associated with rice seeds in India. *Int J Res Pure Appl Microbiol* **3**(1): 25-29.

Basha S and Ulaganathan K 2002. Antagonism of *Bacillus* species (strain BC121) towards *Curvularia lunata. Curr Sci* **82**(12): 1457-63.

Chen W L, Gong H F, Lin F C, Xu J P and Li, D B 1997. The antagonistic activities of *Bacillus subtilis* A30 to rice pathogens. *J Zhejiang Agric Univ* **23**(6): 649-654.

Chhabra R and Vij L 2020. Grain discoloration and its management: an emerging threat to paddy cultivation. *J Pl Dis Prot* **121**: 1-8.

Danquash Q A, Mathur S B and Neergard P 1976. Fungi associated with discoloured rice seeds in Ghana. *Ghana J Agric Sci* **9**(3): 185-87.

Elbeltagy A, Nishioka K, Suzuki H, Sato T, Sato Y I, Morisaki H, Mitsui H and Minamisawa K 2000. Isolation and characterization of endophytic bacteria from wild and traditionally cultivated rice varieties. *Soil Sci Pl Nutri* **46**(3): 617-29.

Ganesan P and Gnanamanickam S S 1987. Biological control of *Sclerotium rolfsii* in peanut by inoculation with *Pseudomonas fluorescens*. *Soil Biol Biochem* **19**(1): 35-38.

Gong Q, Zhang C and Lu F 2014. Identification of bacillomycin D from *Bacillus subtilis* fmbJ and its inhibition effects against *Aspergillus flavus*. *Food Control* **36**(1): 8-14.

ISTA 2003. International rules for seed testing. *Seed Sci Tech* **31**: 61-62.

Kazempour M N and Elahinia S A 2007. Biological control of *Fusarium fuzikuroi*, the causal agent of bakane disease of rice by associated antagonistic bacteria. *Bulgarian J Agric Sci* **13**: 393-408.

Kumar P K M, Gowda S K D, Moudgal R, Kumar K N, Gowda P T K and Vishwanath K 2013. Impact of fungicides on rice production in India. Fungicides- Showcases of integrated plant disease management around the world. Mizuho Nita (edr), Intech open, Croatia, pp. 77-98.

Kumawat G L, Biswas S K and Rajik M 2010. Antagonistic evaluation of *Trichoderma* spp. and their effect on seed germination and growth of paddy seedling. *J Pl Dis Sci* **5**(1): 203-7.

Mandipa R, Dutta S G and Venkata R C 2013. Pseudomonads: Potential biocontrol agents of rice disease. *Res J Agric Forestry Sci* **1**(9): 195.

Naik M R, Akila R and Thiruvudainambi S 2016. Management approaches for brown spot of rice caused by *Bipolaris oryzae. J Farm Sci* **29**(3): 370-76.

Pal K K, Tilak K V B R, Saxena A K, Dey R and Singh C S 2001. Suppression of maize root diseases caused by *Macrophomina phaseolina, Fusarium moniliforme* and *Fusarium graminearum* by plant growth promoting rhizobacteria. *Microbiol Res* **156**: 209-23.

Podile A and Prakash A 1996. Lysis and biological control of *Aspergillus niger* by *Bacillus subtilis* AF 1. *Can J Microbiol* **42**(6): 533-38.

Samanta T T, Das A and Samanta P 2014. Isolation and characterization of *Xanthomonas oryzae* isolates from different regions of Midnapore district of West Bengal and their ecofriendly management by some medicinal plant extracts. *Int J Phytomed* **6**(1): 29.

Sanchez R T, Klu K A Y, Genkinger M J, Lacey V J, Chung T N and Acien N A 2020. Association between rice consumption and risk of cancer incidence in the California teachers study. *Cancer causes and ctrl* **31**: 1129-40.

Sev T M, Khai A A, Aung A and Yu S S 2016. Evaluation of endophytic bacteria from some rice varieties for plant

growth promoting activities. *J Sci Inno Res* **5**(4): 144-48.

Shyamala L and Sivakumar P K 2012. Antifungal activity of Rhizobacteria isolated from rice rhizosphere soil against rice blast fungus *Pyricularia oryzae. Int J Pharm Biol Arch* **3**(3): 692-96.

Suleiman E D and Hussain S S 1984. Pathogenicity and effect on germination caused by *Aspergillus* and *Penicillium* species on wheat, rice, barley and corn. *Pak J Sci Ind Res* **27**(6): 359-62.

Sumangala K, Patil M B, Nargund V B and Ramegowda G 2008. Evaluation of fungicides, botanicals and bioagents against *Curvularia lunata,* a causal agent of grain discolouration in rice. *J Pl Dis Sci* **3**(2): 159-64.

Suresh S R, Yenjerappa S T, Naik M K, Mallesh S B and Kalibavi C M 2013. Studies on cultural and physiological characters of *Xanthomonas oryzae* pv. *oryzae* causing bacterial blight of rice. *Kar J Agric Sci* **26**(2): 214-16.

Tong Y R, Ma Z C, Chen W L and Li D B 1999. Purification and partial characterization of antagonistic proteins from *Bacillus subtilis* B034. *Acta Microbiologica Sinica* **39**(4): 339-43.

Trojanowska K, Stachowiak B, Muzquiz M, Burbano C and Gulewicz K 1998. Opportunities for high quality, healthy and added value crops to meet European demands. Proceedings of the 3rd European conference on grain legumes, Valladoid, Spain. 262p.

Vincent J M 1947. Distortion of fungal hyphae in the presence of certain inhibitors. *Nature* **159**(4051): 850.

Xu J P, Guan J Z, Chen W L, Gong H F and Li D B 1998. Diethyl sulphate induced mutation and screening of *Bacillus subtilis* B826. *J Zhejiang Agric Univ* **24**(6): 595-96.

Zieden E H E 2006. Manipulating endophytic bacteria for biological control to soil borne diseases of peanut. *J Appl Sci Res* **2**(8): 497-502.

Zulfikl E L and Castano J 1991. Effect of grain discoloration in upland rice on some yield loss components. *Int Rice Res Newsltr* **16**(4): 20.