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Variations in Morpho-Cultural Characteristics and Pathogenicity of *Fusarium moniliforme* of Bakanae Disease of Rice and Evaluation of *In Vitro* Growth Suppression Potential of Some Bioagents

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Abstract: Bakanae is one of the important diseases of rice in Bangladesh that causes substantial yield loss every year. We collected thirty isolates of *Fusarium* spp. from bakanae-infected rice plants from different agroecological zones of Bangladesh and investigated the variations in cultural and morphological characteristics and pathogenicity. Diversity was found in cultural characteristics, viz., colony features, phialide, chlamydospore formation, shape, and size of macro- and microconidia. Three variants of *Fusarium* species such as *F. moniliforme*, *F. fujikuroi*, and *F. proliferatum* were identified on PDA media based on their cultural and morphological characteristics. Isolate FM10 (*F. moniliforme*) exhibited the highest disease aggressiveness in developing elongated plants (26.50 cm), the highest number of chlorotic leaves (5.75), and a lower germination percentage. We evaluated different bioagents against the virulent isolate of *F. moniliforme* to develop a rice bakanae disease management approach. Four bioagents, viz., *Trichoderma* spp., *Bacillus subtilis*, *Pseudomonas fluorescens*, and *Achromobacter* spp., were evaluated for growth suppression of *F. moniliforme*. Among the bioagents, *Achromobacter* spp. and *B. subtilis* (BS21) showed 73.54% and 71.61% growth suppression, respectively. The investigation revealed that the application of *Achromobacter* spp. and *B. subtilis* (BS21) would be a potential candidate for effective and eco-friendly management of the bakanae disease of rice.

Keywords: *Fusarium* spp.; bakanae; pathogenicity; bioagents; rice

Citation: Al Amin, A.; Ali, M.H.; Islam, M.M.; Chakraborty, S.; Kabir, M.H.; Khokon, M.A.R. Variations in Morpho-Cultural Characteristics and Pathogenicity of *Fusarium moniliforme* of Bakanae Disease of Rice and Evaluation of *In Vitro* Growth Suppression Potential of Some Bioagents. *Bacteria* **2024**, *3*, 1–14. https://doi.org/10.3390/bacteria3010001

Academic Editors: Marika Pellegrini, Beatriz E. Guerra-Sierra, Debasis Mitra and Bart C. Weimer

Received: 4 November 2023
Revised: 13 January 2024
Accepted: 24 January 2024
Published: 29 January 2024

1. Introduction

Rice (*Oryza sativa* L.) is a prominent staple crop that serves as a significant food product for global populations, with a special emphasis on Asian societies. It provides sustenance for almost 60% of the global population [1]. Approximately 90% of global rice production and consumption exists in Asian regions [2]. Bangladesh is now the third largestrice-producing country in the world after China and India [3]. The total area of rice in Bangladesh is about 11.7 million hectares, with a production of 37.60 million metric tons [4]. The rice crop has a significantly diminished average yield as a consequence of the occurrence of specific illnesses. Rice is affected by a total of 50 diseases, encompassing 21 fungal, 6 bacterial, 12 viral, 4 nematode-related, and 7 other diseases and disorders [5]. These are the major issues responsible for the low yields in Bangladesh. Minor diseases such as false smut, bakanae, sheath rot, and grain discoloration, which were previously ignored, are now posing a severe danger to rice production [6].

Bakanae disease, caused by *Fusarium moniliforme* (anamorph of *Gibberella fujikuroi* Sawada),has recently emerged as a significantly important disease in Asia and other

rice-growing countries of the world [7]. In Bangladesh, bakanae disease has been progressively increasing in prevalence, resulting in both qualitative and quantitative crop losses. Rice yield suffers a regular loss of 20–50% in Japan [8], 40% in Nepal [9], and 10–50% in Bangladesh [10] due to *F. moniliforme*. The first symptoms of bakanae disease are etiolation and aberrant elongation in affected plants through the production of gibberellic acid. Infected plants are taller than healthy plants and light yellowish in color. Roots form at the affected plants' lower nodes. In the advanced stage, a white or light pinkish fungus develops on the lower nodes, and rotting of the foot region occurs, which leads to a few grains of poor quality or the complete death of infected plants [6,11]. Moreover, this fungus produces a diversity of mycotoxins comprised mainly of fumonisin B1 (FB1) during the progression of growth and incursion of rice grains [12]. FB1 is not only responsible for significant economic losses but has also been correlated with high occurrences of liver and esophageal cancer in numerous areas of the world [13].

The establishment of sustainable rice agricultural practices can be attained by the use of novel and enhanced cultivars, alongside the implementation of modern disease management strategies including resistant varieties, cultural practices, and biological and chemical control. All of these strategies have varying degrees of effectiveness in combating diseases. The only way to control bakanae diseases is to treat the seeds with chemical fungicides. However, this is expensive and harmful to both plants and the environment. Biological control is cost-effective and eco-friendly, and it is the most sustainable long-term solution [14]. *Trichoderma* spp., *Pseudomonas* spp., *Achromobacter* spp., and *Bacillus* spp. Have potential antagonist effects against phytopathogens [15]. Among them, *P. fluorescens*, *B. cereus*, and *Trichoderma* spp. isolates have an excellent potential to be used as biocontrol agents of *F. fujikuroi* in rice [16,17]. The capacity of these organisms to colonize roots and their beneficial interactions with plants can lead to an efficient defense against the disease, since they can trigger host defense responses against *Fusarium* spp. attack. In the present study, we evaluated the potential efficacy of bio-agents to suppress the rice bakanae disease causal organism *F. moniliforme* in *in vitro* conditions. The potential bioagents of the present investigation can be applied in the field for controlling bakanae disease.

2. Materials and Methods

2.1. Experimental Site

The experiments were carried out at the Laboratory of Bio-signaling, Bio-active compounds, and Bio-formulation of the Department of Plant Pathology and Professor Golam Ali Fakir Seed Pathology Centre (SPC), Bangladesh Agricultural University, Mymensingh-2202, during the period from January 2019 to December 2019. The laboratory experiment was laid out in a Completely Randomized Design (CRD) with three replications.

2.2. Isolation, Purification, and Identification of the Pathogen

The diseased plant parts and soils around the infected root of bakanae disease were collected from different regions of Bangladesh (Table 1). Blotter incubation and soil dilution methods were followed to isolate *Fusarium* spp. from the infected plant parts of rice [18]. The infected roots of the plants were cut into small pieces of 5 mm length, with some healthy parts. Three layers of blotter papers (Whatman No. 1) were soaked in distilled water and placed at the bottom of plastic petri dishes. The roots were surface sterilized with 10% NaOCl solution for 30 s and subsequent washing with sterilized water three times to remove unwanted microorganisms from the surface of the infected parts. Then, 4–5 small pieces of infected plant roots were placed in petri dishes. Afterward, petri dishes were kept in an incubator at 25 ± 1 °C. After 7 days of incubation, the colony of *Fusarium* spp. was examined under a compound microscope, and pure culture was created by successive culture [7]. In the case of the soil dilution method, soil samples were air-dried and ground with mortar and pestle. One gram of soil sample was mixed into 9 mL distilled water, and then subsequent dilution series were prepared for isolating the fungus. One drop from each dilution was poured into separate 0.2% water agar media in petri dishes. After 7 days

of incubation, the colony of fungi was examined under the microscope, and further pure culture was created [19–21] (Figure 1).

Table 1. Isolates of *Fusarium* spp. collected from different agroecological zones of Bangladesh.

Sl. No.	Isolate Name	Season	Host	District	Village	AEZ
1	FM3	Aus	BRRI dhan 27	Mymensingh	Babuakhali	AEZ 9
2	FM4	Aus	BRRI dhan 27	Mymensingh	Sutiakhali	AEZ 9
3	FM5	Aus	BRRI dhan 27	Mymensingh	Beltuli	AEZ 9
4	FM6	Aus	BRRI dhan 27	Mymensingh	Kurutoli	AEZ 9
5	FM7	Aman	BRRI dhan 49	Mymensingh	BoroBabuakhali	AEZ 9
6	FM8	Aman	BRRI dhan 49	Mymensingh	Curkhai	AEZ 9
7	FM9	Aman	BRRI dhan 49	Mymensingh	Boro Bilar par	AEZ 9
8	FM10	Aman	BRRI dhan 34	Mymensingh	Bagnabari	AEZ 9
9	FM11	Aman	BRRI dhan 34	Mymensingh	Fulpori	AEZ 9
10	FM12	Aman	BRRI dhan 34	Mymensingh	Chanakandi	AEZ 9
11	FM13	Aman	BRRI dhan 34	Mymensingh	Vobakandi	AEZ 9
12	FS16	Boro	BRRI dhan 28	Mymensingh	Bedkanda	AEZ 9
13	FS18	Boro	BRRI dhan 28	Mymensingh	Kewatkhali	AEZ 9
14	FS19	Boro	BRRI dhan 28	Sirajganj	LahiriMohanpur	AEZ 4
15	FS20	Boro	BRRI dhan 28	Sirajganj	Caksa	AEZ 4
16	FS21	Boro	BRRI dhan 28	Sirajganj	Kadapara	AEZ 4
17	FS22	Boro	BRRI dhan 28	Sirajganj	Dohukula	AEZ 4
18	FS23	Boro	BRRI dhan 28	Sirajganj	Boropangasi	AEZ 4
19	FS24	Boro	BRRI dhan 28	Sirajganj	Srekola	AEZ 4
20	FS25	Boro	BRRI dhan 28	Sirajganj	Dukuria	AEZ 4
21	FS26	Boro	BRRI dhan 28	Dhaka	Salna	AEZ 4
22	FS29	Boro	BRRI dhan 28	Dhaka	Salna	AEZ 4
23	FS31	Boro	BRRI dhan 28	Dhaka	Gazipur	AEZ 28
24	FS32	Boro	BRRI dhan 29	Dhaka	Gazipur	AEZ28
25	FS34	Boro	BRRI dhan 29	Dhaka	BSMRAU	AEZ 28
26	FD35	Boro	BRRI dhan 29	Dhaka	BSMRAU	AEZ 28
27	FD41	Boro	BRRI dhan 29	Dhaka	SAU	AEZ 19
28	FD45	Boro	BRRI dhan 29	Dhaka	SAU	AEZ 19
29	FD47	Boro	BRRI dhan 29	Dhaka	Savar	AEZ8
30	FD48	Boro	BRRI dhan 29	Dhaka	Savar	AEZ8

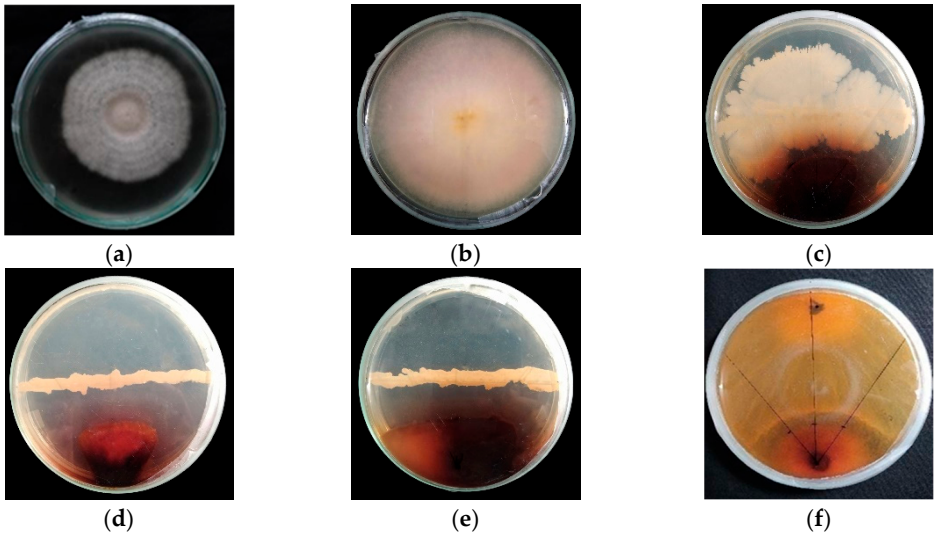

Figure 1. Growth suppression ability of different bioagents against *Fusarium moniliforme*. (**a**) Pure culture of *Fusarium moniliforme*. (**b**) Control. (**c**) *Bacillus subtilis*. (**d**) *Pseudomonas fluorescens*. (**e**) *Achromobacter* spp. (**f**). *Trichoderma* spp.

2.3. Cultural and Morphologic

Mycelial discs (5 mm diameter) of 7-day-old cultures of *Fusarium* spp. of each isolate were transferred to the center of the PDA culture medium. The culture plates were incubated at 25 ± 1 °C for 7 days in an incubator. Colony color, substrate color, phialide, and chlamydospore formation were observed, which developed on the PDA medium from thirty isolates of *Fusarium* spp. Morphology in terms of shape, size, and septation of macro- and microconidia of thirty isolates of *Fusarium* spp. on PDA medium was measured and recorded. Ten-day-old cultures were considered for morphological variability. With the help of a compound microscope, observations of variation in conidial dimension were recorded [22].

2.4. Pathogenicity Test

A pathogenicity test was conducted by seed inoculation assay. The high-yielding rice cultivar BRRI dhan29 was used to test the pathogenicity. The surface of the seeds was disinfected by immersion in 70% ethanol for 1 min, then transferred to 1% sodium hypochlorite solution for 1 min and rinsed three times consecutively in sterile distilled water. Seeds were then left to dry inside the airflow cabinet. Suspension of fungal inoculum was prepared from a 15-day-old culture of *F. moniliforme* that was flooded with sterile water and scraped with a sterile spatula. The resulting suspensions were filtered through two layers of sterile cotton lint, and the concentrations of conidial suspensions were determined with a hemocytometer and adjusted to a concentration of 2×10^6 spores/mL in sterile distilled water. Thirty rice seeds were soaked in 10 mL of inoculum suspension for 18 h at room temperature. Control seeds were soaked in sterile water only. Inoculated and control seeds were then sown in small plastic pots (three pots per isolate and ten seeds per pot) containing an autoclaved mixture of soil and sand at a ratio of 3:1. Fifteen days after inoculation, shoot length (cm), no. of chlorotic leaves, and germination percentage were assessed [22,23]. The seedlings were observed for symptoms of bakanae, as a number of slender and chlorotic, elongated leaves were observed, and a number of plants showed crown rot and produced roots on the lower nodes [24].

2.5. In Vitro Evaluation of Bioagents to Suppress the Growth of the Target Pathogen of Bakanae Disease of Rice

2.5.1. Multiplication of Bioagents

Bacillus subtilis, *Pseudomonas fluorescens*, *Achromobacter* spp., and *Trichoderma* spp. isolates used in the study were obtained from the laboratory of Bio-signaling, Bio-active compounds, and Bio-formulation of the Department of Plant Pathology, Bangladesh Agricultural University. *Bacillus subtilis* and *Achromobacter* spp. isolates were streaked on nutrient agar media, and *Pseudomonas fluorescens* isolates were streaked on King's B media in glass petri dishes. Then, the petri dishes were kept in an incubator at 28 °C for 24 h. On the other hand, *Trichoderma* spp. was cultured on sterilized PDA plates. The plates were then incubated at 26 °C for 7 days. A 5 mm culture block from the plate of *Trichoderma* spp. was transferred to the new PDA plates to maintain pure culture and stored in a refrigerator at 4 °C for further studies.

2.5.2. *In Vitro* Growth-Suppressing Ability of Bioagents against *Fusarium moniliforme*

The growth suppression ability of *B. subtilis*, *P. fluorescens*, *Achromobacter* spp., and *Trichoderma* spp. isolates against *F. moniliforme* was examined by dual-culture technique [25] and incubation at 26 °C for 8–10 days. Three replications were maintained for each isolate. Observations on the width of the inhibition zone and radial mycelial growth at 9 and 12 days after incubation of the test pathogen were recorded, and percent inhibition of pathogen growth was calculated using the formula proposed by Vincent [26]:

$$\text{Percent inhibition (I)} = (C - T/C) \times 100$$

where C = mycelial growth of the pathogen in control; T = mycelial growth of the pathogen in the dual-culture plate.

2.6. Disease Assessment

Disease assessment (Table 2) was done following the scale reported by Scherm et al. [27]

Table 2. The disease intensity of bakanae is rated using a 0–4 scale. The scale spans over 5 classes [27].

Rating Number	Reaction Description
0	No disease symptoms
1	Normal growth but leaves beginning to show yellowish green color
2	Abnormal growth; elongated, thin, and yellowish-green leaves; seedlings also shorter or taller than normal
3	Abnormal growth; elongated, chlorotic, thin, and brownish leaves; seedlings also shorter or taller than normal
4	Seedlings with fungal mass on the surface of infected plants or dead plants

2.7. Statistical Analyses

Statistical analyses were performed by R statistical software (http://www.R-project.org, accessed on 25 October 2023) to find out the significance of the differences among the bio-agents. ANOVA was performed by the Agricola R package [28], and mean differences were adjudged by Tukey's HSD test.

3. Results

3.1. Cultural and Morphological Variation in Fusarium spp.

Different isolates of the pathogens associated with bakanae disease of rice were identified on PDA media as *Fusarium moniliforme*, *F. fujikuroi*, and *F. proliferatum* based on their cultural and morphological characteristics (Tables 3 and 4). *F. moniliforme* is responsible for producing gibberellic acid, which causes abnormal elongation of plants, resulting in bakanae disease of rice. *F. fujikuroi* (FM3, FM11, FM13, FS16, FS19, FS20, FS21, FS26, FS34, FD35, FD47, FD48) isolates were identified based on characteristics like monophialide and simple polyphialide structure. Obovoid with flattened base, oval (0–1 septate), allantoid pyriform (rarely) microconidia (5.31–13.59 µm × 1.44–12.96 µm) were present. Macroconidia (57.35–118.51 µm × 11.17–23.05 µm) were falcate to almost straight and slender in shape (1–3 septation). *F. moniliforme* isolates (FM5, FM6, FM7, FM8, FM9, FM10, FM12, FS18, FS23, FD41) were characterized by simple and branch monophialide structures. The size of microconidia was 4.49–13.89 µm × 4.78–10.88 µm, and the shape was obovoid with a flattened base, oval to long oval, and elliptical. Macroconidia were falcate to straight, with three to five septate and a size of 54.68–177.33 µm × 11.29–18 µm. *F. proliferatum* isolates (FM4, FS22, FS24, FS25, FS29, FS31, FS32, FD45) have monophialide and polyphialide structures. Microconidia were obovoid with a flattened base, pyriform, and allantoid in shape, and sizes were 2.65–21.74 µm × 1.75–18.40 µm. Macroconidia were falcate to almost straight, slender, with one to four septate and a size of 57.07–254.98 µm × 15.81–33.10 µm. Both *F. moniliforme* and *F. fujikuroi* produce a large amount of toxins and metabolites that cause pigmentation in PDA media. The colors vary from light orange or pinkish to deep brown and sometimes grayish violet. On the other hand, *F. proliferatum* shows pinkish white to grayish black stripes.

Table 3. Variation in colony characteristics of *Fusarium* spp. isolates on PDA media.

Sl. No.	Isolates	Colony Features		Phialide	Chlamydospore
		Colony Colors	Substrate Color		
1	FM3	White orange to pale orange, grayish strip present	Orange to light orange, blackish strip	Monophialide and simple polyphialides	absent
2	FM4	White orange to pale orange, grayish strip present	Orange to light orange, blackish strip	Monophialide and polyphialides	absent
3	FM5	Pinkish white to grayish violet	Deep orange to pale orange	Simple and branch monophialide	absent
4	FM6	Pinkish white to grayish violet	Deep orange to pale orange	Simple and branch monophialide	absent
5	FM7	Pinkish white to pale orange	Light orange with grayish strip	Simple and branch monophialide	absent
6	FM8	Pinkish white to pale orange	Deep orange to pale orange, grayish strip	Simple and branch monophialide	absent
7	FM9	Pinkish white to grayish violet	Deep orange to grayish strip	Simple and branch monophialide	absent
8	FM10	Pinkish white to grayish orange	Light orange to grayish strip	Simple and branch monophialide	absent
9	FM11	White orange to pinkish white	Light orange with grayish strip	Monophialide and simple polyphialides	absent
10	FM12	Pinkish white with gray strip	Light orange with grayish strip	Simple and branch monophialide	absent
11	FM13	White orange to grayish violet	Light orange with grayish strip	Monophialide and simple polyphialides	absent
12	FS16	Grayish white to gray	Deep orange, black in center	Monophialide and simple polyphialides	absent
13	FS18	Pinkish white to grayish violet	Pale orange, deep orange in center	Simple and branches monophialide	absent
14	FS19	White to grayish white, gray strip	Solid orange	Monophialide and simple polyphialides	absent
15	FS20	Light orange with grayish strip	Light orange with grayish strip	Monophialide and simple polyphialides	absent
16	FS21	Pinkish white to light brown	Deep and light orange patch	Monophialide and simple polyphialides	absent
17	FS22	Pinkish white	Deep orange to dark black in center	Monophialide and polyphialides	absent
18	FS23	Pale orange to grayish violet	Light orange at periphery and deep orange on center	Simple and branch monophialide	absent
19	FS24	Pinkish white to grayish black, striped	Pale white to pale orange	Monophialide and polyphialides	absent
20	FS25	Pale orange to grayish violet	Dark orange to dark gray	Monophialide and polyphialides	absent
21	FS26	Pinkish white to pale orange	Pale orange	Monophialide and simple polyphialides	absent
22	FS29	Pinkish white, pale orange in center	Dark orange and grayish strip	Monophialide and polyphialides	absent
23	FS31	Pale orange to violet, striped	Dark orange and grayish strip	Monophialide and polyphialides	absent
24	FS32	White, no pigmentation	Faded white	Monophialide and polyphialides	absent
25	FS34	Orange-white to grayish violet	Pale orange to pale white	Monophialide and simple polyphialides	absent
26	FD35	Pinkish white to pale orange	Deep orange on periphery, light in center	Monophialide and simple polyphialides	absent
27	FD41	Pale orange to pinkish white	Deep orange on periphery, light in center	Simple and branch monophialide	absent
28	FD45	Pinkish white and pale orange strip	Deep orange on periphery, light in center	Monophialide and polyphialides	absent
29	FD47	Grayish violet	Pale orange to grayish violet	Monophialide and simple polyphialides	absent
30	FD48	Pale orange to pinkish white	Deep orange on periphery, light in center	Monophialide and simple polyphialides	absent

Table 4. Variation in shape, size, and septation of microconidia and macroconidia of *Fusarium* spp. isolates on PDA media.

Sl. No.	Isolates	Microconidia			Macroconidia	
		Chain	Shape	Size	Shape	Size
1	FM3	Present	Obovoid with flattened base, oval (0−1 septate), allantoid pyriform (rarely)	10.7 µm× 9.14 µm	Falcate to almost straight, slender, 1−3 septate (majority 3 septate)	64.63 µm × 15.43 µm
2	FM4	Present	Obovoid with flattened base, pyriform, allantoid	21.74 µm × 18.40 µm	Falcate to almost straight, slender, 1−4 septate (majority 3 septate)	84.75 µm × 21.83 µm
3	FM5	Present	Obovoid with flattened base, oval to long oval, elliptical, globose (rarely)	9.30 µm × 7.94 µm	Falcate to straight, slender, 3−5 septate (majority 3 septate)	70.10 µm × 16.89 µm
4	FM6	Present	Obovoid with flattened base, oval to long oval, elliptical, globose (rarely)	10.7 µm × 9.33 µm	Falcate to straight, slender, 3−5 septate (majority 3 septate)	64.63 µm × 13.27 µm
5	FM7	Present	Obovoid with flattened base, oval to long oval, elliptical, globose (rarely)	9.30 µm × 8.45 µm	Falcate to straight, slender, 3−5 septate (majority 3 septate)	70.13 µm × 15.43 µm
6	FM8	Present	Obovoid with flattened base, oval to long oval, elliptical, globose (rarely)	13.89 µm × 10.88 µm	Falcate to straight, slender, 3−5 septate (majority 3 septate)	69.67 µm × 17.79 µm
7	FM9	Present	Obovoid with flattened base, oval to long oval, elliptical, globose (rarely)	13.83 µm × 12.83 µm	Falcate to straight, slender, 3−5 septate (majority 3 septate)	88.16 µm × 12.5 µm
8	FM10	Present	Obovoid with flattened base, oval to long oval, elliptical, globose (rarely)	13.76 µm × 10.86 µm	Falcate to straight, slender, 3−5 septate (majority 3 septate)	88.19 µm × 12.5 µm
9	FM11	Present	Obovoid with flattened base, oval (0−1 septate), allantoid pyriform (rarely)	13.59 µm × 12.96 µm	Falcate to almost straight, slender, 1−3 septate (majority 3 septate)	72.03 µm × 12.50 µm
10	FM12	Present	Obovoid with flattened base, oval to long oval, elliptical, globose (rarely)	10.07 µm × 9.34 µm	Falcate to straight, slender, 3−5 septate (majority 3 septate)	64.63 µm × 13.27 µm
11	FM13	Present	Obovoid with flattened base, oval (0−1 septate), allantoid pyriform (rarely)	6.47 µm × 6.47 µm	Falcate to almost straight, slender, 1−3 septate (majority 3 septate)	57.35 µm × 18.98 µm
12	FS16	Present	Obovoid with flattened base, oval (0−1 septate), allantoid pyriform (rarely)	5.83 µm × 5.83 µm	Falcate to almost straight, slender, 1−3 septate (majority 3 septate)	68.59 µm × 23.05 µm
13	FS18	Present	Obovoid with flattened base, oval to long oval, elliptical, globose (rarely)	4.49 µm × 3.24 µm	Falcate to straight, slender, 3−5 septate (majority 3 septate)	56.09 µm × 11.29 µm
14	FS19	Present	Obovoid with flattened base, oval (0−1 septate), allantoid pyriform (rarely)	5.31 µm × 3.92 µm	Falcate to almost straight, slender, 1−3 septate (majority 3 septate)	61.95 µm × 11.17 µm
15	FS20	Present	Obovoid with flattened base, oval (0−1 septate), allantoid pyriform (rarely)	5.58 µm × 5.58 µm	Falcate to almost straight, slender, 1−3 septate (majority 3 septate)	69.19 µm × 12.16 µm
16	FS21	Present	Obovoid with flattened base, oval (0−1 septate), allantoid pyriform (rarely)	12.62 µm × 3.08 µm	Falcate to almost straight, slender, 1−3 septate (majority 3 septate)	61.09 µm × 14.52 µm
17	FS22	Present	Obovoid with flattened base, pyriform, allantoid	11.62 µm × 3.25 µm	Falcate to almost straight, slender, 1−4 septate (majority 3 septate)	245.98 µm × 23.82 µm
18	FS23	Present	Obovoid with flattened base, oval to long oval, elliptical, globose (rarely)	7.50 µm × 5.69 µm	Falcate to straight, slender, 3−5 septate (majority 3 septate)	177.33 µm × 21.88 µm
19	FS24	Present	Obovoid with flattened base, pyriform, allantoid	6.90 µm × 4.53 µm	Falcate to almost straight, slender, 1−4 septate (majority 3 septate)	127.5 µm × 26.64 µm
20	FS25	Present	Obovoid with flattened base, pyriform, allantoid	5.96 µm × 5.90 µm	Falcate to almost straight, slender, 1−4 septate (majority 3 septate)	58.24 µm × 33.10 µm

Table 4. *Cont.*

Sl. No.	Isolates	Microconidia			Macroconidia	
		Chain	Shape	Size	Shape	Size
21	FS26	Present	Obovoid with flattened base, pyriform, allantoid	6.41 µm × 4.54 µm	Falcate to almost straight, slender, 1−3 septate (majority 3 septate)	61.29 µm × 19.03 µm
22	FS29	Present	Obovoid with flattened base, pyriform, allantoid	9.33 µm × 2.73 µm	Falcate to almost straight, slender, 1−4 septate (majority 3 septate)	142.78 µm × 22.45 µm
23	FS31	Present	Obovoid with flattened base, pyriform, allantoid	2.65 µm × 2.65 µm	Falcate to almost straight, slender, 1−4 septate (majority 3 septate)	127.51 µm × 24.40 µm
24	FS32	Present	Obovoid with flattened base, pyriform, allantoid	3.52 µm × 1.75 µm	Falcate to almost straight, slender, 1−4 septate (majority 3 septate)	149.71 µm × 15.81 µm
25	FS34	Present	Obovoid with flattened base, oval (0−1 septate), allantoid pyriform (rarely)	7.14 µm × 1.55 µm	Falcate to almost straight, slender, 1−3 septate (majority 3 septate)	94.29 µm × 17.27 µm
26	FD35	Present	Obovoid with flattened base, oval (0−1 septate), allantoid pyriform (rarely)	8.81 µm × 4.80 µm	Falcate to almost straight, slender, 1−3 septate (majority 3 septate)	65.57 µm × 21.66 µm
27	FD41	Present	Obovoid with flattened base, oval to long oval, elliptical, globose (rarely)	9.54 µm × 4.78 µm	Falcate to almost straight, slender, 1−3 septate (majority 3 septate)	54.68 µm × 18.00 µm
28	FD45	Present	Obovoid with flattened base, pyriform, allantoid	6.27 µm × 5.02 µm	Falcate to almost straight, slender, 1−4 septate (majority 3 septate)	57.07 µm × 18.03 µm
29	FD47	Present	Obovoid with flattened base, oval (0−1 septate), allantoid pyriform (rarely)	9.58 µm × 4.58 µm	Falcate to almost straight, slender, 1−3 septate (majority 3 septate)	118.51 µm × 15.54 µm
30	FD48	Present	Obovoid with flattened base, oval (0−1 septate), allantoid pyriform (rarely)	7.12 µm × 5.54 µm	Falcate to almost straight, slender, 1−3 septate (majority 3 septate)	73.92 µm × 20.87 µm

3.2. Evaluation of Pathogenic Variation in Different Isolates of Fusarium spp.

Thirty isolates of *Fusarium spp.* were subjected to a pathogenicity test to observe the elongated shoot length, number of chlorotic leaves, and germination percentage. The pathogenicity of different isolates was estimated as the degree of disease symptom expression 15 days after sowing (Table 5). The highest shoot length was observed in FM10 (26.50 cm), and the lowest length was recorded in FD35 (12.00 cm). The highest number of chlorotic leaves was observed in FM11 (5.75) isolates, followed by FM4, FM9, FM10, FS19, FS21, FS24, FS34, and FD45. At 15 DAS, the lowest germination percentage was observed in the FD35 isolate, which was statistically similar to the FM10 isolate. As such, the presence of a heavy pathogen dose of *Fusarium* spp. seed germination hampers plants drastically, and the FM10 (*F. moniliforme*) isolate was selected for further research experiments, as it showed the maximum aggressiveness in bakanae disease formation.

Table 5. Assessing pathogenicity of different isolates of *Fusarium moniliforme* collected from different AEZs of Bangladesh by artificial inoculation in plants grown in pots (15DAS).

Sl. No.	Isolates of *F. moniliforme*	Shoot Length (cm)	No. of Chlorotic Leaves	Germination (%)
1	FM3	21.25 d–g	3.50 c–g	67.86 a–c
2	FM4	21.00 d–h	5.25 ab	75.00 ab
3	FM5	22.75 b–e	1.00 ij	64.29 a–d

Table 5. *Cont.*

Sl. No.	Isolates of *F. moniliforme*	Shoot Length (cm)	No. of Chlorotic Leaves	Germination (%)
4	FM6	18.00 g–k	1.75 hi	67.86 a–c
5	FM7	21.75 c–f	1.75 hi	64.29 a–d
6	FM8	21.50 d-f	3.00 d–h	60.71 a–e
7	FM9	25.50 ab	4.75 a–c	50.00 b–g
8	FM10	26.50 a	4.50 a–d	39.29 d–g
9	FM11	25.00 a–c	5.75 a	60.71 a–e
10	FM12	20.50 e–i	4.00 b–f	75.00 ab
11	FM13	20.50 e–i	3.00 d–h	50.00 b–g
12	FS16	19.75 e–j	2.25 g–i	57.14 a–f
13	FS18	21.00 d–h	3.25 c–h	28.57 g
14	FS19	24.00 a–d	4.25 a–e	57.14 a–f
15	FS20	19.25 f–j	1.00 ij	35.71 e–g
16	FS21	18.00 g–k	4.75 a–c	53.57 b–g
17	FS22	21.75 c–f	4.00 b–f	46.43 c–g
18	FS23	19.25 f–j	2.75 e–h	35.71 e–g
19	FS24	21.75 c–f	4.75 a–c	57.14 a–f
20	FS25	19.25 f–j	2.75 e–h	32.14 fg
21	FS26	15.25 kl	1.75 hi	42.86 c–g
22	FS29	17.00 jk	3.00 d–h	39.29 d–g
23	FS31	12.50 l	3.75 b–g	46.43 c–g
24	FS32	18.00 g–k	3.25 c–h	39.29 d–g
25	FS34	18.50 f–k	4.50 a–d	32.14 fg
26	FD35	12.00 l	2.50 f–i	28.57 g
27	FD41	15.25 kl	3.50 c–g	60.71 a–e
28	FD45	17.75 h–k	4.75 a–c	57.14 a–f
29	FD47	17.25 i–k	2.75 e–h	32.14 fg
30	FD48	19.25 f–j	3.50 c–g	50.00 b–g
31	Control	13.50 l	0.00 j	82.14 a
CV (%)		12.55	32.80	35.36
Level of significance		*	*	*

DAS = days after sowing, CV = co-efficient of variations. Here, values in the column having a similar letter (s) are statistically identical (Tukey's HSD test at $p < 0.05$). * = 5% level of significance.

3.3. In Vitro Growth Suppression of Fusarium moniliforme by Bacillus subtilis

Eight *Bacillus subtilis* isolates were evaluated against *F. moniliforme* in *in vitro* conditions following a dual-culture plate technique on a PDA medium. The highest (54.64 mm) mycelial growth was observed on the control plate. The highest inhibition (71.61%) of mycelial growth over the control was observed in the BS21 isolate, and the lowest (18.69%) mycelial growth inhibition was found in the BS31 isolate (Table 6). Therefore, the BS21 isolate showed the most potent inhibition against *F. moniliforme* (Figure 1c).

Table 6. *In vitro* evaluation of different isolates of *Bacillus subtilis* for suppressing the growth of *Fusarium moniliforme*.

Sl. No	Isolates of *B. subtilis*	Radial Mycelial Growth (mm)			% Growth Inhibition over Control
		9 DAI	12 DAI	Mean	
1	BS10	15.85 b	18.89 c	17.37	68.21
2	BS17	18.77 b	24.11 c	21.44	60.76
3	BS21	12.34 b	18.67 c	15.51	71.61
4	BS26	14.52 b	19.89 c	17.21	68.50
5	BS27	18.17 b	24.33 c	21.25	61.11
6	BS31	39.75 a	49.11 b	44.43	18.69

Table 6. *Cont.*

Sl. No	Isolates of *B. subtilis*	Radial Mycelial Growth (mm)			% Growth Inhibition over Control
		9 DAI	12 DAI	Mean	
7	BS41	17.26 b	20.45 c	18.86	65.48
8	BS8	17.23 b	21.11 c	19.17	64.92
9	Control	46.05 a	63.22 a	54.64	-
CV (%)		18.37	20.07	-	-
Level of significance		*	*	-	-

DAI = days after incubation, CV = co-efficient of variations, BS = *Bacillus subtilis*. Here, values in the column having a similar letter (s) are statistically identical (Tukey's HSD test at $p < 0.05$). * = 5% level of significance.

3.4. In Vitro Growth Suppression of Fusarium moniliforme by Pseudomonas fluorescens

The application of eight *P. fluorescens* isolates was assessed against *F. moniliforme* in in vitro conditions. The highest (57.77 mm) mycelial growth was observed in the PF9 isolate, followed by the control plate (54.64 mm). The highest percent inhibition (58.64%) of mycelial growth over the control was observed in the case of isolate PF7. The lowest (4.52%) mycelial growth inhibition was found in isolate PF18 (Table 7). Among all the isolates of *Pseudomonas fluorescens*, PF7 showed the strongest inhibition against *F. moniliforme* (Figure 1d).

Table 7. *In vitro* evaluation of different isolates of *Pseudomonas fluorescens* for suppressing the growth of *Fusarium moniliforme*.

Sl. No	Isolates of *P. fluorescens*	Radial Mycelial Growth (mm)			% Growth Inhibition over Control
		9 DAI	12 DAI	Mean	
1	PF10	23.50 bc	25.00 cd	24.25	55.61
2	PF11	49.27 a	61.45 a	55.36	No inhibition
3	PF18	47.67 a	56.67 a	52.17	4.52
4	PF2	39.62 a–c	50.00 ab	44.81	18
5	PF5	38.78 a–c	48.89 a–c	43.84	19.77
6	PF7	20.63 c	24.56 d	22.60	58.64
7	PF8	21.01 c	27.22 b–d	24.12	55.86
8	PF9	50.42 a	65.11 a	57.77	No inhibition
9	Control	46.05 ab	63.22 a	54.64	-
CV (%)		35.65	30.07	-	-
Level of significance		*	*	-	-

DAI = days after incubation, CV = co-efficient of variations, PF = *Pseudomonas fluorescens*, No inhibition = growth more than control. Here, values in the column having a similar letter (s) are statistically identical (Tukey's HSD test at $p < 0.05$). * = 5% level of significance.

3.5. In Vitro Growth Suppression of Fusarium moniliforme by Achromobacter spp.

Achromobacter spp. Was tested in vitro against *F. moniliforme* using a dual-culture plate technique on a PDA medium. Significant growth suppression (73.54%) of *F. moniliforme* by *Achromobacter* spp. was found (Table 8 and Figure 1e).

Table 8. In vitro evaluation of *Achromobacter* spp. for suppressing the growth of *Fusarium moniliforme*.

Sl. No	Isolate of *Achromobacter* spp.	Radial Mycelial Growth (mm)			% Growth Inhibition over Control
		9 DAI	12 DAI	Mean	
1	*Achromobacter* spp.	12.57 b	16.34 b	14.46	73.54
2	Control	46.05 a	63.22 a	54.64	-
CV (%)		11.80	9.58	-	-
Level of significance		*	*	-	-

DAI = days after incubation, CV = co-efficient of variations. Here, values in the column having a similar letter (s) are statistically identical (Tukey's HSD test at $p < 0.05$). * = 5% level of significance.

3.6. In Vitro Growth Suppression of Fusarium moniliforme by Trichoderma spp.

Invitro evaluation of *Trichoderma* spp. against *F. moniliforme* was performed following a dual-culture plate technique on a PDA medium. A total of 69.84% radial mycelial growth inhibition of *F. moniliforme* by *Trichoderma* spp. was recorded (Table 9 and Figure 1f).

Table 9. Invitro evaluation of *Trichoderma* spp. for suppressing the growth of *Fusarium moniliforme*.

Sl. No	Isolate of *Trichoderma* spp.	Radial Mycelial Growth (mm)			% Growth Inhibition over Control
		9 DAI	12 DAI	Mean	
1	*Trichoderma* spp.	14.07 b	18.89 b	16.48	69.84
2	Control	46.05 a	63.22 a	54.64	-
CV (%)		9.23	2.97	-	-
Level of significance		*	*	-	-

DAI = days after incubation, CV = co-efficient of variations. Here, values in the column having a similar letter (s) are statistically identical (Tukey's HSD test at $p < 0.05$). * = 5% level of significance.

4. Discussion

Rice bakanae disease is emerging as the most destructive disease for rice cultivation in Bangladesh. It causes greater yield losses across the rice-growing regions of the country. The pathogen primarily survives in seeds but is also known to survive in the soil [8]. Based on cultural, morphological, and pathogenicity tests, we identified the causal agent of bakanae disease of rice as *F. moniliforme*. Pandey et al. [7] also noted that *F. moniliforme* causes bakanae disease in rice in Nepal. These *Fusarium* species could differ with geographic as well as climatic variations [8]. Leslie et al. [29] identified *Fusarium* spp. using the morphological structure and different size and shape of the conidia. According to Nirenberg et al. [30], morphological features of the fungal isolates were assessed based on the size and the shape of micro- and macroconidia. Different isolates showed different colors of their colony in the PDA medium. The fungal isolates were identified on PDA media as *F. moniliforme*, *F. fujikuroi*, and *F. proliferatum* based on their colony features, phialide, and chlamydospore formation, as well as shape, septation, and size of macro- and microconidia. Among them, *F. moniliforme* is responsible for producing large quantities of gibberellic acid (GA3),which causes the seedling to have abnormal elongation and results in bakanae disease of rice. Sunder and Satyavir [31] reported that the isolates of *F. moniliforme* varied greatly in producing GA3 in liquid culture. There were several numbers of *F. moniliforme*, *F. fujikuroi*, and *F. proliferatum* isolated. Therefore, it was necessary to find the most pathogenic strain among the isolates. For this, pathogenicity tests of 30 isolates of *Fusarium* species were carried out using rice plants under a net house. Among them, the FM10 (*F. moniliforme*) isolate caused the highest shoot elongation in plants (26.50 cm), the highest number of chlorotic leaves (5.75), and the lowest germination percentage due to its maximum pathogenic virulence. Bashyal et al. [32] reported that Pusa Basmati 1121 rice was susceptible to bakanae disease and exhibited more abnormal elongation, rotting, and shrinkage of leaves. Therefore, effective strategies are essential to manage bakanae disease. This disease is controlled by chemical fungicides, but these are also severe environmental

hazards [33]. To overcome this problem, bio-agents are used to suppress bakanae diseases and work through their production of various secondary metabolites [34]. In this study, we have used four bio-agents against *F. moniliforme*,viz., *Trichoderma* spp., *Bacillus subtilis*, *Pseudomonas fluorescens*, and *Achromobacter* spp., to control bakanae disease in plants of the next generation. Among the bio-agents, *Achromobacter* spp. and *Bacillus subtilis* (BS21) performed excellently and showed 73.54% and 71.61% inhibition, respectively. Sarwar et al. [34] reported that *B. subtilis* NH-100 and *Bacillus* sp. NH-217 and their surfactin exhibit remarkable antagonistic activity against bakanae disease in Super Basmati rice caused by *F. moniliforme. Trichoderma* spp. produced good inhibitory results under in vitro conditions, and it may represent an important biocontrol agent to control the bakanae disease of rice [6]. Similar observations were also made by Pal et al. [35] and Patkowska [15]. Gupta et al. [8] revealed that some strains of *Trichoderma*, *Pseudomonas*, and *Bacillus* have anti-fungal effects against bakanae disease. A similar finding was investigated by Jing and Suga [17]. Therefore, the introduction of biocontrol agents may be an innovative treatment capable of suppressing bakanae disease to be used instead of chemical treatment.

5. Conclusions

For sustainable and eco-friendly disease management, it is crucial to find an advanced and effective management technique superior to traditional practices. In modern agriculture, it is possible to use bio-agents that protect plants against phytopathogens. *Trichoderma* spp., *Pseudomonas* spp., *Achromobacter* spp., and *Bacillus subtilis* act as bio-agents that exhibit remarkable antagonistic activity against the bakanae disease of rice. The present study showed that *Achromobacter* spp. and *Bacillus subtilis* could be effective biocontrol agents against the bakanae disease in rice and should be incorporated into strategies for disease management. However, further research is necessary to replicate the experiment in field conditions in different AEZs for at least two years before a recommendation is made to farmers.

Author Contributions: A.A.A.: Experimental set up, treatment application, writing of original draft; M.H.A.: Analysis and interpretation of data; M.M.I.: Data collection and tabulation, development of graphs and tables; S.C. and M.H.K.: Review and editing of manuscript; M.A.R.K.: Design of experiment, finalization of manuscript, supervision of experiment. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Ministry of Science and Technology (MoST) Project (Project no.: 2019/38/MoST) in Bangladesh.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The manuscript contains all the necessary data.

Acknowledgments: This research work was a part of the MS research of the first author, financially supported by the National Science and Technology (NST), Ministry of Science and Technology, Govt. of the People's Republic of Bangladesh.

Conflicts of Interest: The authors declare that there are no conflict of interest.

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