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RESEARCH

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Management of bacterial wilt (*Ralstonia solanacearum*) of brinjal using *Bacillus cereus*, *Trichoderma harzianum* and *Calotropis gigantea* consortia in Bangladesh

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Abstract

Background Bacterial wilt caused by *Ralstonia solanacearum* is a devastating disease of brinjal in Bangladesh. The study was targeted to evaluate the bacterial wilt management ability of microbial consortia composed of isolated and identified native *Bacillus cereus, Trichoderma harzianum* and *Calotropis gigantea* for the first time in Bangladesh.

Results Twenty bacterial strains were isolated from the rhizosphere of the brinjal plant following serial dilution method. Among the strains, HSTUB 17 showed maximum zone of inhibition (1.5 ± 0.1 cm) against *R. solanacearum* in the dual culture method. Molecular characterization using 16 s rRNA partial coding sequence revealed HSTUB 17 as *B. cereus*. Consortia composed with the identified *B. cereus* HSTUB 17 (10^8 CFU ml^{-1} @ 5 ml/plant), previously isolated *T. harzianum* (@5 mm size of four mycelial disk/plant) and aqueous leaf extracts of *C. gigantea* (1:1, w/v basis @ 40 ml/plant) were applied in the root zone following soil drenching method and found to reduce bacterial wilt incidence by 74.87, 66.67 and 66.67% at 30, 50 and 70 days after transplanting, respectively, in comparison with plants received only *R. solanacearum* (10^8 CFU ml^{-1} @ 5 ml/plant). The single application of *B. cereus* HSTUB 17, *T. harzianum* and *C. gigantea* also minimized wilt incidence by 21.16–37.34, 33.33 and 21.48–28.14%, respectively, on all the days of observations. The consortia of *B. cereus* HSTUB 17, *T. harzianum* and *C. gigantea* also resulted in maximum plant height (56.67 cm), the number of branches/plants (10.33), the number of fruits/plants (8.33) and fruit yield (25.56 ton/ha) in comparison with the plant exposed to *R. solanacearum* only.

Conclusion The findings of the study revealed the potentiality of consortia composed of *B. cereus* HSTUB 17, *T. harzianum* and *C. gigantea* for the eco-friendly management of bacterial wilt of brinjal for the first time in Bangladesh.

Keywords Brinjal, *Ralstonia solanacearum*, Bio-agents, Consortia, Wilt disease, Yield

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Background

Eggplant or brinjal (*Solanum melongena* L.) belongs to the family Solanaceae and is one of the important vegetable crops ranked second after potato in Bangladesh (BBS 2021). The annual production of brinjal (587 metric tons) in Bangladesh is far low in comparison with other brinjal-producing countries like India, China and Japan (BBS 2021). Various factors such as environmental and edaphic conditions, insect pests and diseases play significant role in the reduced yield of brinjal. About 12 diseases were reported associating with brinjal, which considered as the major concerning issue in Bangladesh for the lower production of brinjal. Among the diseases, bacterial wilt caused by *Ralstonia solanacearum* is the most destructive in the field conditions and reported to contribute about 10–90% yield loss in Bangladesh (Nishat et al. 2015). *Ralstonia solanacearum* infects the plant's roots from transplanting to harvesting by colonizing the vascular system, blocking the translocation of water and nutrients (Sharma and Sharma 2014).

For the management of bacterial wilt, various tactics including crop rotation, use of resistant varieties, grafting with disease-free wild rootstock, soil fumigation, chemicals, etc. have been reported (Islam et al. 2014). Farmers of Bangladesh traditionally use chemicals as the least option for controlling such kinds of devastating diseases even prior to any suggestions from the experts. Hence, using chemicals is now becoming a mammoth threat to the environment, human health and the development of resistance to plant pathogens (Nishimoto 2019). Therefore, researchers around the world are trying to explore natural-based alternative ways of controlling wilt disease without chemicals.

Integration of naturally available plant disease management tools including beneficial microbes, such as *Bacillus*, *Pseudomonas*, *Trichoderma*, along with different medicinal plants has gained momentum for their cost-effectiveness, durability and environmental safety nature (Lahlali et al. 2022). The biocontrol agents, *Trichoderma* as well as *Bacillus* spp., have long been well proved for their efficacy in controlling numerous soilborne plant pathogens including *R. solanacearum* (Konappa et al. 2018). Moreover, *Trichoderma* and *Bacillus* also enhanced plant growth and yield in various crops (Islam et al. 2022). Again, *Calotropis gigantea* (akondo) also showed disease suppressing and plant growth ability, as it possessed various antimicrobial compounds like flavonoids, alkaloids, saponins and tannins (Shrivastava et al. 2013). However, the combination of various biocontrol agents as consortia demonstrated the superior crop disease management efficacy and enhanced crop growth in comparison with their single application (Jahagirdar et al. 2021). So far, limited or no work on the management of bacteria wilt of brinjal using the consortia of *Bacillus*, *Trichoderma* and *Calotropis* has been reported in Bangladesh. Therefore, the present study was designed to evaluate the efficacy of the consortia composed of *B. cereus*, *T. harzianum* and *C. gigantea* for the eco-friendly management of bacterial wilt of brinjal.

Methods

Isolation and identification of *R. solanacearum*

Ralstonia solanacearum, the causal agent of bacterial wilt of brinjal, was isolated from the wilted brinjal plant following Kelman (1954). In brief, wilted brinjal plants were brought to the laboratory, washed vigorously in running tap water, cut into small pieces and surface-sterilized using 0.1% $HgCl_2$ for 1 min followed by three times washing with double-distilled sterilized water (ddsH_2O). The cut pieces were then transferred into a beaker containing ddsH_2O for 5 min to get milky, white bacterial exudates. Bacterial suspension from the beaker was then directly streaked on the Petri plates containing sterilized and solidified nutrient agar (NA) (meat extract, 10 g; peptone, 10 g; NaCl, 1.5 g; agar, 15 g; adjusted to 1000 ml) and incubated at 28 ± 2 °C for 2 days. Finally, the isolate was re-streaked on triphenyl tetrazolium chloride (TTC or TZC) medium to obtain the single colony. Naked eye observation was carried out for the morphological characters of the isolates.

Pathogenicity of the isolated *R. solanacearum*

Pathogenicity test of the isolated *R. solanacearum* was performed by inoculating it to healthy brinjal seedlings (BARI Hybrid Brinjal-4) following Gutarra et al. (2017). The roots of 2-week-old brinjal seedlings were damaged with a sterile scissor and inoculated with 4 ml of overnight cultured bacterial suspension (10^8 cfu/ml). A total of six seedlings was used for the pathogenicity test, where control was also maintained without the inoculation of bacterial suspension.

Isolation and purification of *Bacillus* spp.

Beneficial bacterial strains were isolated from the rhizosphere soil of brinjal plants (BARI Hybrid Brinjal-4) following serial dilution method (Prashanthi et al. 2021). In brief, 1 g soil was suspended in 9 ml ddsH_2O and diluted up to 10^{-6}. From the diluted suspension, a total of 100 µl was poured onto Petri plates containing NA medium and incubated at 28 ± 2 °C for 48 h. The developed single colony was further streaked onto a new NA containing Petri plates and preserved in a refrigerator at 4 °C for further use.

In vitro screening of the isolated *Bacillus* spp. against *R. solanacearum*

The antibacterial efficacy of the isolated *Bacillus* spp. against *R. solanacearum* was carried out on NA medium following the agar well diffusion method (Lemessa and Zeller 2007). In brief, a total of 100 µl of overnight cultured *R. Solanacearum* (10^8 cfu/ml) was spread on NA medium, followed by the making of three holes (9 mm) by using a sterile cork borer. Thirty µL beneficial bacterial suspensions (10^7 cfu/ml) of each strain was then, added to each hole and incubated at 28 ± 2 °C for 48 h. The growth inhibition *R. solanacearum* in response to the beneficial bacterial strains was measured as the radius of the inhibition zone (cm).

Morphological and biochemical characterization of *Bacillus* spp.

The beneficial bacterial strains (*Bacillus* spp.) that showed antibacterial efficacy against *R. solanacearum* were selected for morphological study. Twenty-four-h-old bacterial colonies were cultured on an NA medium and used to observe the colony color, shape, etc. using the naked eye and microscopic observation (Goodfellow et al. 2012).

Potassium hydroxide (KOH) test

A loopful of 3-day-old bacterial colony was mixed with two drops of 3% KOH solution on a glass slide and stirred for 10 s in a circular motion, and the formation of slime threads was observed by raising 1 cm from the surface using a toothpick (Suslow et al. 1982).

Catalase oxidase test

A single drop of 3% solution of hydrogen peroxide (H_2O_2) was mixed with a loopful of 3-day-old bacterial culture on a glass slide, and the production of gas bubbles was observed with the naked eye (Reiner 2010).

Starch hydrolysis test

Bacterial strains were cultured on NA medium containing 0.2% soluble starch (w/v) and incubated for 2 days at 28 ± 2 °C. After heavy growth of the strains, IKI solution (Iodine 1 g, potassium iodide 2 g, distilled water 100 ml) was added to the plates and examined development of a clear zone around the colony just after 30 s (Sands 1990).

Tobacco hypersensitivity test

The bacterial suspension (10^8 cfu/ml) in deionized water was injected into the intercellular space of the tobacco leaves with the help of a hypodermic syringe. Negative control was maintained by infiltrating the leaves with deionized water, and leaf interactions were noticed at 24 h after infiltration (Klement et al. 1990).

Potato soft rot test

Potato soft rot test for the strains was conducted by placing sterilized potato slices (7–8 mm thickness) in a sterilized Petri plate containing moistened and sterile filter paper (Whatman no. 1). Spore suspension of each bacterial strain was then, sprayed on the sliced potato and observed for 48 h for the development of rotting symptoms (Lelliott and Stead 1987). Only sterile water sprayed on potato slices was treated as a control.

Molecular characterization of the isolated *Bacillus* spp.

The bacterial strain, namely HSTUB 17, which showed the highest zone of inhibition against *R. solanacearum*, was selected for the molecular characterization using 16S rRNA partial coding sequence. Genomic DNA of HSTUB 17 was extracted by using a homogenizer (Pro Scientific) and an automated DNA extractor (model: Maxwell 16, Promega, USA), quantified with a NanoDrop spectrophotometer (model: ND2000, Thermo Scientific, USA) and kept at − 20 °C until further use. Tow universal primers, viz. 27 forward: 5′ AGAGTTTGATCMTGGCTCAG 3′ and 1492 Reverse: 5′ CGGTTACCTTGTTACGAC TT 3′, were used to amplify the DNA. PCRs were carried out in a 20 µl reaction containing DNA template (1 µl of 25–65 ng/µl) mixed with 10 µl hot start green master mix (Buffer, dNTPs, $MgCl_2$ and Taq polymerase; origin: Promega, USA), 1 µl of each primer (10–20 pMol) and PCR grade water. The reactions mixture was placed in a thermocycler (Gene Atlas, model: G2, origin: Astec, Japan) with an initial denaturation profile for 3 min at 95 °C, denaturation for 30 s at 95 °C, annealing for 30 s at 48 °C and extension for 90 s at 72 °C, followed by thirty-five cycles with final extension for 5 min at 72 °C. The PCR product was then, loaded on 1.0% agarose gel along with 1 Kb DNA ladder (Promega, USA; Horizontal, model: mini, origin: CBS Scientific, USA) and documented (Alpha Imager, model: Mini, origin: Protein Simple, USA). PCR products were then, purified using SV gel and PCR cleanup system (Promega, USA; Centrifuge, model: Kitman24, origin: Tomy, Japan) and sequenced following Sanger Sequencing by Apical Scientific-Malaysia.

Constructions of phylogenetic tree

The homology comparison of the 16S rRNA gene sequence of HSTUB 17 was retrieved by running the sequenced data through the BLASTN on the NCBI database (http://www.ncbi.nlm.nih.gov/Blast.cgi). The phylogenetic tree was constructed using the MEGA 11 software package following the neighbor-joining method (Saitou and Nei 1987; Tamura et al. 2021).

Collection of *T. harzianum*

Trichoderma harzianum was collected from the Department of Plant Pathology, HSTU, which was previously isolated and identified. The antagonist was subcultured and kept in a refrigerator at 4 °C until further use.

Collection and preparation of leaf extracts of *C. gigantea*

Aqueous extracts of *C. gigantea* were prepared by using fresh leaves (Ul-Haq et al. 2014). In brief, collected fresh leaves were washed in running tap water and air-dried in the shed. Totally, 100 g dried leaves was dipped in 100 ml ddsH$_2$O (1:1, w/v basis), blended using an electric blender, filtered using a double-layered fine muslin cloth and kept in a refrigerator at 4 °C until further use.

Management of bacterial wilt of brinjal using the identified *B. cereus* HSTUB 17, *T. harzianum* and *C. gigantea*

A field study was carried out at the central research field, HSTU, Dinajpur, Bangladesh (25° 13′ N latitude and 88° 23′ E longitudes), during the Rabi season (Nov.-March) of 2019–2020. Brinjal seeds (BARI Hybrid Brinjal-4) were collected from Bangladesh Agricultural Research Institute (BARI), Joydebpur, Gazipur, surface sterilized with 10% sodium hypochlorite (NaOCl) solution for 2 min, washed with ddsH$_2$O for 3 times and air-dried. Fifty seeds were sown in each of the earthen pots ($22\times14\times6$ cm^3) containing sterilized soil and well-decomposed cow dung (1:2). Twenty-five-day-old seedlings were transplanted to the field (@4 plants/plot) with an individual plot size of 1.5 m × 1.0 m, plot to plot distance of 50 cm. Nine treatment combinations, viz. control (without *R. solanacearum* or any bio-agents) (Ctrl); negative control (only *R. solanacearum*) (NCtrl); *R. solanacearum* and *B. cereus* HSTUB 17 (Bc); *R. solanacearum* and *T. harzianum* (Th); *R. solanacearum* and *C. gigantea* (Cg); *R. solanacearum, B. cereus* HSTUB 17 (Bc) and *C. gigantea* (Cg); *R. solanacearum*, *B. cereus* HSTUB 17 (Bc) and *T. harzianum* (Th); *R. solanacearum, B. cereus* HSTUB 17 (Bc), *T. harzianum* (Th) and *C. gigantea* (Cg); and *R. solanacearum* and streptocycline (Strp). All the treatments were replicated thrice, following Randomized Complete Block Design (RCBD). The bio-agents were applied, 7 days after transplantation (DAT) as follows: both *B. cereus* and *R. solanacearum* (10^8 cfu/ml) @ 5 ml/plant; four mycelial disk (5 mm dia.) of *T. harzianum* at the root zone of each plant; spraying of streptocycline @ 0.5 g/l of water; *C. gigantea* leaf extract @ 40 ml/plant. The intercultural operations were maintained throughout the growing season as and when necessary. Bacterial wilt incidence (%) was recorded at 30, 50 and 70 DAT (Song et al. 2004):

$$\text{Percentage of disease incidence (PDI)} = \frac{\text{Number of wilted plants per plot}}{\text{Total number of plants per plot}} \times 100$$

Plant height (cm), the number of branches/plants and the number of leaves/plants were also recorded at 30, 50 and 70 DAT. The number of fruits/plants was recorded at 70 and 90 DAT, and yield (ton/ha) was estimated from each of the plots.

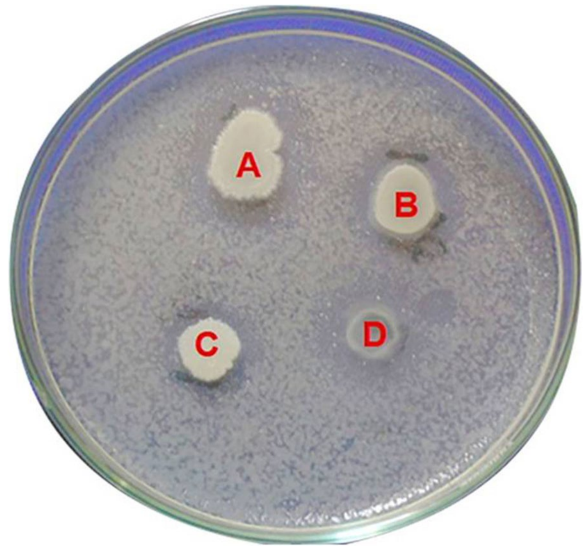

Fig. 1 In vitro screening of *Bacillus* spp. against *Ralstonia solanacearum*; **A** HSTUB 14; **B** HSTUB 17; **C** HSTUB 18; **D** HSTUB 12

Statistical analysis

Data collected on wilt incidence and agronomic attributes were analyzed using the statistical package "R" (version 4.1.2.). The means of all the treatments were computed by DMRT (Duncan multiple range test) at 5% level of probability (Gomez and Gomez 1984).

Results

Identification of *R. solanacearum*

The purified colonies of *R. solanacearum* on TTC medium showed the light red or pink color with a characteristic red center and whitish margin. The isolated *R. solanacearum* produced characteristics wilt symptoms in the inoculated brinjal plant.

Screening of *Bacillus* sp. against *R. solanacearum*

A total of 20 bacterial strains were isolated from the rhizosphere of brinjal plants. Among the isolated strains, four strains, namely HSTUB 12, HSTUB 14, HSTUB 17 and HSTUB 18, showed antibacterial efficacy against *R. solanacearum* (Fig. 1). However, HSTUB 17 gave the highest zone of inhibition (1.5 ± 0.1 cm), followed by HSTUB 14 (1.0 ± 0.2), HSTUB 12 (0.9 ± 0.1) and HSTUB 18 (0.7 ± 0.2) (Table 1).

Table 1 Morphological and biochemical characterization of the isolated bacterial strains (*Bacillus* spp.)

Serial no	Bacterial strains	Zone of inhibition (cm) against *Ralstonia solanacearum*	Biochemical tests					Inference
			KOH*	Starch Hydrolysis*	Soft Rot*	Catalase Oxidase*	Tobacco Hypersensitivity*	
1	HSTUB 1	–	–	–	–	–	–	–
2	HSTUB 2	–	–	–	–	–	–	–
3	HSTUB 3	–	–	–	–	–	–	–
4	HSTUB 4	–	–	–	–	–	–	–
5	HSTUB 5	–	–	–	–	–	–	–
6	HSTUB 6	–	–	–	–	–	–	–
7	HSTUB 7	–	–	–	–	–	–	–
8	HSTUB 8	–	–	–	–	–	–	–
9	HSTUB 9	–	–	–	–	–	–	–
10	HSTUB 10	–	–	–	–	–	–	–
11	HSTUB 11	–	–	–	–	–	–	–
12	HSTUB 12	0.9 bc ± 0.1	"–" ve	"–" ve	"–" ve	"+" ve	"+" ve	*Bacillus* sp.
13	HSTUB 13	–	–	–	–	–	–	–
14	HSTUB 14	1.0 b ± 0.2	"–" ve	"–" ve	"–" ve	"+" ve	"+" ve	*Bacillus* sp.
15	HSTUB 15	–	–	–	–	–	–	–
16	HSTUB 16	–	–	–	–	–	–	–
17	HSTUB 17	1.5 a ± 0.1	"–" ve	"–" ve	"–" ve	"+" ve	"+" ve	*Bacillus* sp.
18	HSTUB 18	0.7 c ± 0.2	"–" ve	"–" ve	"–" ve	"+" ve	"+" ve	*Bacillus* sp.
19	HSTUB 19	–	–	–	–	–	–	–
20	HSTUB 20	–	–	–	–	–	–	–

*"+" ve = Positive reaction, "–" ve = Negative reaction

Identification of *Bacillus* sp.

All the four strains, namely HSTUB 12, HSTUB 14, HSTUB 17 and HSTUB 18, were found to produce creamy yellowish or whitish color colonies, flat or somewhat convex with rough edges on NA medium. Again, all the strains also showed positive reactions to catalase oxidase and tobacco hypersensitivity tests, and negative responses to KOH, starch hydrolysis and soft rot tests (Fig. 2; Table 1). Further, totally 1500-bp PCR-amplified DNA was obtained from HSTUB 17 which showed maximum similarity and close relationship with *B. cereus* strain K1M36 (accession no. MW559327) and *B. cereus* strain K1M20 (accession no. MW559293) by both BLASTN search and phylogenetic tree analysis (Fig. 3).

Efficacy of the consortia composed of *B. cereus* HSTUB 17, *T. harzianum* and *C. gigantea* against bacterial wilt

In comparison with plants inoculated with *R. solanacearum* only, the consortia composed of *B. cereus* HSTUB 17, *T. harzianum* and *C. gigantea* resulted maximum reduction of bacterial wilt incidence (74.87, 66.67 and 66.67%), followed by consortia of *B. cereus* HSTUB 17 and *C. gigantea* (49.37, 55.56 and 43.70%); consortia of *B. cereus* HSTUB 17 and *T. harzianum* (49.37, 44.90 and 44.90%) at 30, 50 and 70 DAT, respectively (Table 2). Plants that received no bio-agents or only *B. cereus* HSTUB 17 or *T. harzianum* or *C. gigantea* also reduced wilt incidence by 13.07–37.34, 22.22–33.33 and 11.11–28.14%, respectively, in comparison with plants inoculated with *R. solanacearum* only (Table 2).

Along with the suppression of wilt disease, the consortia of *B. cereus* HSTUB 17, *T. harzianum* and *C. gigantea* significantly increased plant height (28.93–56.67 cm) and the number of branches/plants (6.08–10.33) at all date of observations in comparison with the *R. solanacearum* inoculated plant (27.60–49.67 cm and 4.75–6.50). At 70 and 90 DAT, the highest number of fruits/plants (6.58 and 8.67) was also recorded in response to the application of the consortia composed of *B. cereus* HSTUB 17, *T. harzianum* and *C. gigantean*. Likewise, the number of fruits and maximum yield (25.56 ton/ha) were also obtained with the consortia composed of *B. cereus* HSTUB 17, *T. harzianum* and *C. gigantean,* followed by the consortia of *B. cereus* HSTUB 17 and *T. harzianum* (17.78 ton/ha); and consortia of *B. cereus* HSTUB 17 and *C. gigantea* (16.67 ton/ha) (Table 3).

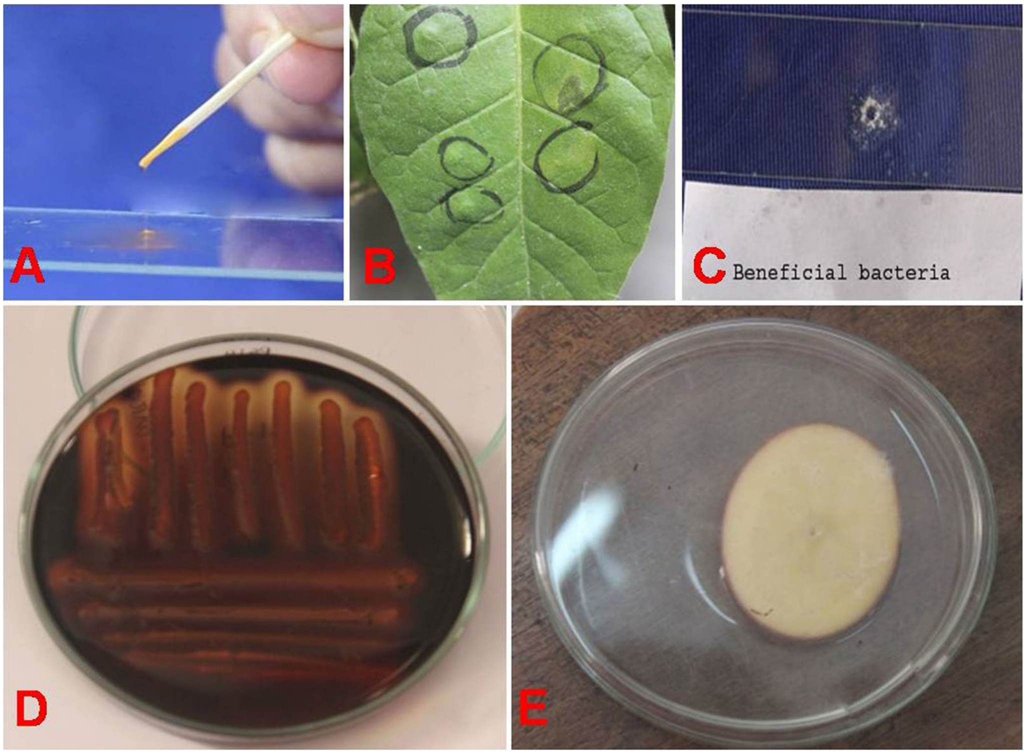

Fig. 2 Biochemical characterization of *Bacillus* spp. **A** Potassium hydroxide (KOH) test; **B** tobacco hypersensitivity test; **C** catalase oxidase test; **D** starch hydrolysis test; **E** potato soft rot test

Discussion

The isolated and purified *R. solanacearum* produced characteristics light red or pink colony on TTC or TZC (Kelman 1954), which after inoculation produced wilt symptoms in brinjal plants (Akter et al. 2021). After getting entrance to the plant, *R. solanacearum* colonizes in the xylem vessel which eventually blocks the translocation of water from the root to the upper parts of the plant and cause wilt symptoms (Ingel et al. 2022). For the controlling of wilt diseases, 20 rhizosphere bacteria were isolated and screened them against *R. solanacearum* under in vitro. Among the isolates, *B. cereus* HSTUB 17 suppressed the growth of *R. solanacearum* with a maximum zone of inhibition. Beneficial bacteria, viz. *Bacillus*, *Pseudomonas*, etc., possess several antimicrobial secondary metabolites including volatile compounds, enzymes and siderophores which are responsible for the broad-spectrum antibacterial efficacy against various fungal and bacterial pathogens (Iqbal et al. 2021). However, the potential strains obtained in this study were assumed as *Bacillus* spp., as they produced characteristics of creamy yellowish or whitish color colonies with rough edges (Al-Saraireh et al. 2015). The potential strains also showed positive reactions in catalase oxidase test by producing gas bubbles and hypersensitive response in tobacco hypersensitivity test and hence were considered as *Bacillus* spp. (Goodfellow et al. 2012). More than 90% similarity of around 1500-bp gene sequence of *B. cereus* HSTUB 17 with the deposited partial gene sequence of *B. cereus* in NCBI and its phylogenetic tree analysis confirm the isolate as *B. cereus* (Moussa et al. 2022).

The isolated *B. cereus* HSTUB 17 along with *T. harzianum* and *C. gigantea* either alone or in various combinations demonstrated remarkable suppression of bacterial wilt disease of brinjal in field conditions in the present study. The bio-agents as consortia not only reduced the disease, but also enhanced different agronomic traits of the brinjal plant including yield. *Bacillus* spp. and *Trichoderma* spp. have long proved their efficacy in controlling various plant diseases caused by a wide range of plant pathogens including *R. solanacearum* (Zhou et al. 2021). Besides, the use of plant extracts including *C. gigantea* leaf extracts was reported to show antimicrobial effects, when applied against numerous plant diseases (Abo-Elyousr et al. 2022). In contrast to the single application, the consortia of bio-agents showed maximum suppression of wilt diseases along with enhanced agronomic attributes. The consortia of beneficial bacteria including *Bacillus*, *Pseudomonas*, etc. with *Trichoderma* have been found to suppress diversified diseases in contrast to their single

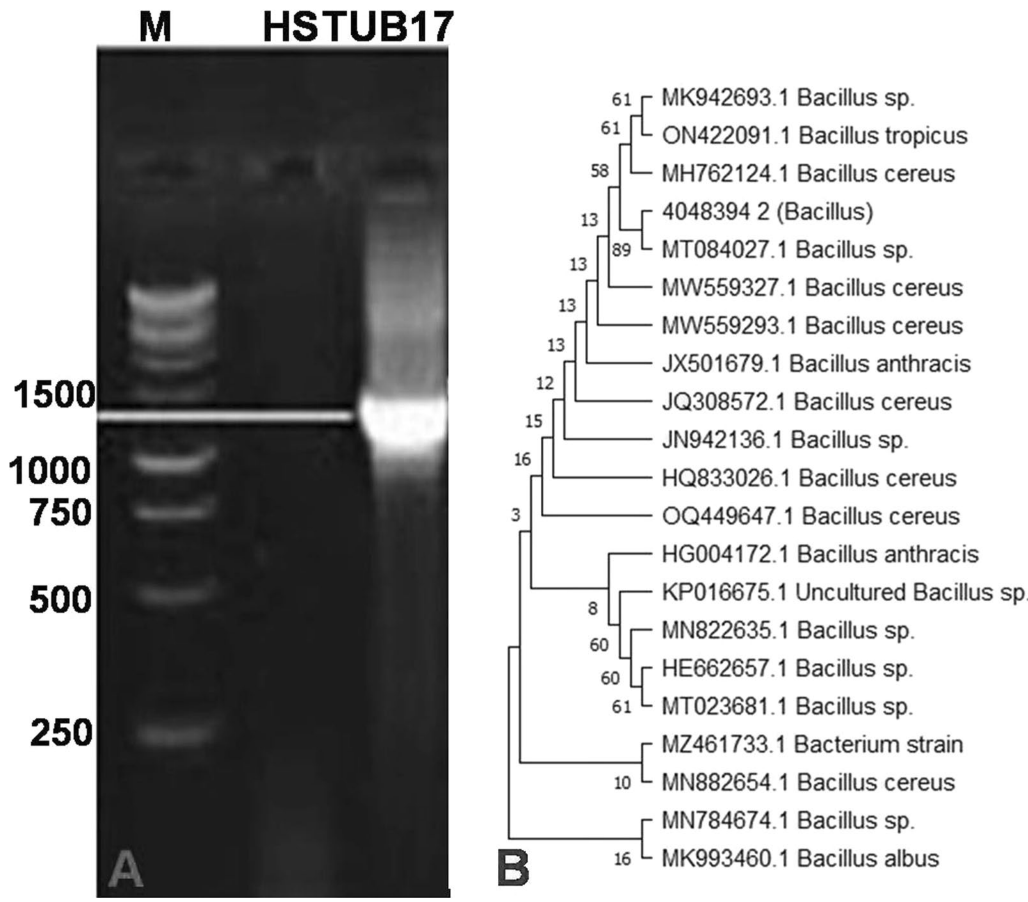

Fig. 3 **A** Gel electrophoresis of the amplified DNA obtained from *Bacillus cereus* HSTUB 17. M; marker; **B** phylogenetic tree of *B. cereus* HSTUB 17 using 16S rRNA, showing names of related bacterial species

Table 2 Efficacy of consortia of *Bacillus cereus* HSTUB 17, *Trichoderma harzianum* and *Calotropis gigantea* on wilt incidence (%) of brinjal at different days of observations

Treatments	Wilt incidence (%)					
	30 DAT*	Reduction (%) over NCtrl	50 DAT*	Reduction (%) over NCtrl	70 DAT*	Reduction (%) over NCtrl
Ctrl	69.83b	13.07	70.00b	22.22	80.00b	11.11
NCtrl	80.33a	–	90.00a	–	90.00a	–
Cg	50.67d	36.92	60.00c	33.33	70.67c	21.48
Bc	63.33c	21.16	60.00c	33.33	70.67c	21.48
Th	50.33d	37.34	60.00c	33.33	64.67d	28.14
Bc + Cg	40.67e	49.37	40.00e	55.56	50.67e	43.70
Bc + Th	40.67e	49.37	50.00d	44.90	50.00e	44.90
Bc + Th + Cg	20.16f	74.87	30.00f	66.67	30.00 ± 0.f	66.67
Strp	10.00g	87.55	30.00f	66.67	30.00f	66.67
LSD ($p \leq 0.05$)	3.13		3.81		2.16	
CV (%)	3.82		4.04		2.09	

*Means in the columns with different letters are significantly different ($p < 0.05$); Ctrl = Without *R. solanacearum* or any bio-agents; NCtrl = Only *Ralstonia solanacearum*; Cg = *R. solanacearum* and *C. gigantea*; Bc = *R. solanacearum* and *B. cereus* HSTUB 17; Th = *R. solanacearum* and *T. harzianum*; Strp = *R. solanacearum* and streptocycline

Table 3 Efficacy of consortia of *Bacillus cereus* HSTUB 17, *Trichoderma harzianum* and *Calotropis gigantea* on different agronomic traits of brinjal at different days of observations

Treatments	Plant height (cm)			Number of branches/plants			Number of fruits/plants		Yield (ton/ha)*
	30 DAT*	50 DAT*	70 DAT*	30 DAT*	50 DAT*	70 DAT*	70 DAT*	90 DAT*	
Ctrl	28.17 ± 0.29cd	44.67 ± 0.58f	51.33 ± 0.58e	6.17 ± 0.58d	7.50 ± 0.50e	7.83 ± 0.29e	2.67 ± 0.58f	4.00 ± 0.50e	9.56 ± 2.69cd
NCtrl	27.60 ± 0.53d	42.67 ± 0.58g	49.67 ± 1.53e	4.75 ± 0.66d	5.33 ± 1.04f	6.50 ± 0.50f	2.76 ± 0.24f	3.75 ± 0.25e	6.22 ± 0.77d
Cg	28.93 ± 0.12bc	45.50 ± 0.50ef	51.33 ± 0.58e	6.08 ± 0.88e	8.25 ± 0.25d	8.50 ± 0.50de	4.17 ± 0.29e	4.67 ± 0.58de	13.56 ± 1.02bc
Bc	29.17 ± 0.29bc	46.43 ± 0.40de	53.00 ± 1.00d	6.67 ± 0.76cd	8.33 ± 0.29cd	9.17 ± 0.29cd	4.33 ± 0.29de	4.92 ± 0.88cde	14.44 ± 1.93b
Th	28.97 ± 1.00bc	46.67 ± 0.58d	54.33 ± 0.58cd	6.75 ± 0.43cd	8.50 ± 0.50cd	8.83 ± 0.29d	4.75 ± 0.25cde	5.67 ± 0.62cd	15.78 ± 4.44b
Bc + Cg	29.77 ± 0.20b	47.6 ± 0.58c	54.00 ± 1.00cd	7.00 ± 1.00cd	9.00 ± 0.00bc	9.83 ± 0.76bc	5.16 ± 0.76cd	6.00 ± 1.00c	16.67 ± 3.33b
Bc + Th	29.67 ± 0.58b	48.50 ± 0.50bc	55.00 ± 1.00c	7.50 ± 0.50bc	9.33 ± 0.58ab	9.83 ± 0.76bc	5.58 ± 0.52bc	7.33 ± 0.58b	17.78 ± 2.77b
Bc + Th + Cg	29.73 ± 0.64b	49.17 ± 0.76ab	56.67 ± 0.58b	8.17 ± 0.76ab	9.83 ± 0.29a	10.33 ± 0.29ab	6.58 ± 0.52a	8.67 ± 0.58ab	25.56 ± 1.93a
Strp	30.83 ± 0.29a	49.63 ± 0.63a	58.67 ± 1.15a	9.00 ± 0.50a	10.00 ± 0.00a	10.75 ± 0.43a	6.17 ± 0.29ab	8.33 ± 0.58a	25.56 ± 1.93a

*Means in the columns with different letters are significantly different ($p < 0.05$) according to LSD tests; Ctrl = Without *R. solanacearum* or any bio-agents; NCtrl = Only *Ralstonia solanacearum*; Cg = *R. solanacearum* and *C. gigantea*; Bc = *R. solanacearum* and *B. cereus* HSTUB 17; Th = *R. solanacearum* and *T. harzianum*; Strp = *R. solanacearum* and Streptocycline

use (Chaudhary et al. 2023). Again, the combination of two *Bacillus* strains showed elicited activities of superoxide dismutase and peroxidases during the management of soilborne diseases of tomato and pepper in comparison with their single application (Jetiyanon 2007). The secretion or presence of various inhibitory metabolites such as indole-3-acetic acid, β-1-3-glucanase, siderophores and gibberellins in *Trichoderma*; 2,3-butanediol, cellulose, acetoin, antibiotics and lipopeptides in *Bacillus*; and flavonoids, phenols, polysaccharide terpenes and saponins in *C. gigantean* might be responsible for triggering plant growth and yield with reduced plant disease (Albayrak 2019). *Trichoderma* and *Rhizobium* consortia already demonstrated enhanced uptake of nitrogen and phosphorous during the management of soilborne diseases in chickpeas (Rudresh et al. 2005). Moreover, compatible microbes in consortia may show better performance than the single use due to the exposure of several additives or synergistic tactics with numerous modes of action, viz. competition, mycoparasitism, induced systemic resistance, etc. (Sarma et al. 2015).

Conclusion

The rhizosphere of crop plants is the harbor of microbial antagonists that assist plants with systematic resistance along with enhanced growth and yield. The present study explored the ability of consortia composed with a native *Bacillus cereus*, *T. harzianum* and *C. gigantea* for the management of bacterial wilt of brinjal in field conditions. Compared to the single use, consortia of the selected bio-agents resulted in maximum reduction of wilt incidence (%). The consortia not only reduced the disease, but it also enhanced plant height, the number of branches and yield of the brinjal plant. However, the findings of the study explore the probability of the use of *B. cereus* HSTUB 17, *T. harzianum* and *C. gigantea* as consortia for the successful management of wilt of brinjal in Bangladesh. In addition, the consortia might also facilitate the absorption of various nutrients by the plant which has a direct effect on the minimization of chemical fertilizers. In the future, the persistence of the bio-agent in soil needs to be assessed for their better performance as consortia.

Abbreviations

CFU	Colony-forming unit
NA	Nutrient agar
ddsH$_2$O	Double-distilled sterilized water
TTC	Triphenyl tetrazolium chloride
RCBD	Randomized complete block design
DAT	Days after transplantation
PDI	Percentage of disease incidence
DMRT	Duncan multiple range test

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Author contributions

MUQ conducted the experiment, collected and analyzed the data, and drafted the manuscript. MMI wrote the manuscript. SkMMH analyzed the data. MEKC reviewed the manuscript. MMH designed the experiment, reviewed and edited the manuscript.

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Availability of data and materials
All datasets on which conclusions of the study have been drawn are presented in the main manuscript.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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