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RESEARCH

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Effect of endophytic *Bacillus* and arbuscular mycorrhiza fungi (AMF) against *Fusarium* wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici*

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Abstract

Background: *Fusarium* wilt of tomato caused by *Fusarium oxysporum* f. sp. *lycopersici* (FOL) is a serious disease that causes significant economic losses in tomato production. Seventeen endophytic *Bacillus* isolates from tomato roots of Meghalaya were tested for antagonistic and plant growth promotion activities. Dominating arbuscular mycorrhiza fungi (AMF) spores were isolated from the rhizosphere soils of tomato grown in Meghalaya. The effect of different combinations of AMF and endophytic *Bacillus* on *Fusarium* wilt severity and growth of tomato plant under pot and field conditions was studied.

Results: The endophytic *Bacillus* isolates ERBS51 and ERBS10 showed a maximum inhibition against FOL, with 58.43 and 55.68%, respectively, in a dual culture experiment. ERBS51 and ERBS10 were identified as *Bacillus velezensis* and *Bacillus* sp., respectively, based on 16s rRNA sequencing. Both isolates were found positive for iturin A, surfactin, bacillomycin D, protease, cellulase, pectinase, alpha-amylase, siderophore, ammonia production and $ZnCO_3$ solubilization. *Funneliformis mosseae* and *Glomus fasciculatum* were the dominating AMF species in tomato rhizosphere of Meghalaya. The result of pot and field experiments revealed that out of all the treatments, combination of *Funneliformis mosseae* + *Glomus fasciculatum* + *Bacillus velezensis* + *Bacillus* sp. was shown to be the best in reducing the severity of *Fusarium* wilt to 77.44 and 66.74%, respectively. *F. mosseae* + *G. fasciculatum* + *B. velezensis* + *Bacillus* sp. also recorded the highest in most growth attributes and yield.

Conclusions: Endophytic *Bacillus* (*B. velezensis* and *Bacillus* sp.) and AMF (*F. mosseae* and *G. fasciculatum*) were safe and effective biocontrol agents against *Fusarium* wilt of tomato.

Keywords: *Bacillus*, Arbuscular mycorrhizal fungi, Meghalaya, Isolates, Field experiments

Background

Tomato is affected by various constraints like biotic factors such as diseases caused by fungi, bacteria, virus, nematodes, insect pests and some abiotic factors during its growth stages that result in loss of yield (Liu and Wang 2020). Among the various plant pathogens attacking tomato, *Fusarium oxysporum* f. sp. *lycopersici* is an important pathogen that has been documented to occur and cause damage to tomato globally by decreasing the yield in both open field and greenhouse (McGovern 2015). *Fusarium* wilt is a soil-borne fungal pathogen capable of surviving in the soil and crop debris for many years and has been known to enter the plant through wounds on roots.

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The pathogen's soil-borne nature and its ability to invade vascular tissue makes it challenging to manage this disease (Ajilogba and Babalola 2013). The use of fungicides and resistant varieties are the main practices for management of *Fusarium* wilt of tomato, but due to several environmental problems, achieving sustainable agriculture requires avoidance of chemical pesticides. Such chemical fungicides tend to accumulate in the food chain and have a detrimental impact on soil microorganisms (Lopez-Aranda et al. 2016). Similarly, breeding for resistance also has its limitations and difficulty when there is no *F. oxysporum* resistance genes available (Ploetz 2015). Plant root diseases can be controlled by manipulation of microbes or by introducing them in soil (Vazquez et al. 2000), representing a natural, safe, effective and durable alternative to the use of pesticides. The battle to manage *Fusarium* wilt has prompted the adoption of biological control, and numerous researches have been conducted by using biocontrol in managing this disease. Soil-borne pathogens like *Fusarium* wilt pathogen can be reduced by several soil microbes which have antagonistic action (Jamil et al. 2021).

Among the various endophytes associated with plants without causing negative impact to the host, the bacterial genus, *Bacillus* is one of the most frequently occurring endophyte that has been used as a biocontrol agent (Nandhini et al. 2012). *Bacillus* species ability to produce endospores renders them resistant to severe environmental conditions, making them a good choice for biocontrol agent. The antagonistic activity of *Bacilli* maybe due to production of siderophore and extracellular metabolites (Miljaković et al. 2020). Additionally, *Bacillus* can also trigger induced systemic resistance (ISR), thereby improving the host plants response against the attacking bacterial, fungal and viral pathogens (Miljaković et al. 2020). An Arbuscular mycorrhizal fungi (AMF) forms symbiotic relationship with roots and colonizes the roots of the most plant species and benefits each other (Wang and Qiu 2006). They can enhance plants resistance to infection of pathogen and stress (Sawers et al. 2018). They also produce a defence mechanism known as mycorrhiza-induced resistance (MIR), which works against different attackers like pathogenic fungi, generalist chewing insects and necrotrophs (Nguvo and Gao 2019).

It is fascinating to look into the synergistic impacts of both AMF and *Bacillus* when used in combination to improve plant disease resistance and growth since both AMF and *Bacillus* already have their own different positive effect on plants. The control of *Fusarium* wilt of tomato by the application of AMF and endophytic *Bacillus* has been reported by several workers (Hashem et al. 2021). This appears to be tremendous antagonistic potential for AMF and endophytic *Bacillus* as a promising way of controlling plant diseases as an environmentally safe alternative. Meghalaya, like the rest of the north-east, has a long history of organic farming, free of chemicals. This gives it the advantage of easily transitioning from chemical to sustainable organic farming. So, biological control can certainly be an alternative strategy for management of *Fusarium* wilt in tomato growing areas of Meghalaya. Taking in considerations of the above situation, the present investigation was undertaken to study the biocontrol potentials of endophytic *Bacillus* under in vitro conditions, to isolate dominating AMF spores and to study their effect as single or combined inoculations against *Fusarium* wilt of tomato as an eco-friendly way of control along with its growth-enhancing effect on tomato.

Methods

Isolation of *Fusarium* wilt pathogen

In order to isolate *Fusarium* wilt pathogen, the root and stem tissues of wilted tomato plant samples obtained from a farmer's field were washed under tap water to remove the soil particles. The infected tissues were cut into 5–10 mm long pieces, followed by surface sterilization for 1 to 2 min in 2% sodium hypochlorite solution and washed twice in sterile distilled water. These surface sterilized tissues were placed on potato dextrose agar (PDA) plates and incubated at 28 ± 2 °C for 7–10 days. The fungal culture was purified using Rangaswami's (2005) hyphal tip cut technique. Potato dextrose agar (PDA) slants were used to maintain the pure cultures.

Pathogenicity test and inoculum preparation

Pathogenicity test was performed using Devi and Srinivas (2012) root dip technique. Three-week-old tomato seedlings were chosen and 2–4 cm root tip were cut and immersed in one-week-old FOL conidial suspension of 10^6 spores/ml for 30 min. The inoculated seedlings were transplanted into a disposable polyvinyl chloride container containing 1:1 soil and sand mixture. The plant showing complete wilting symptoms was reisolated according to Koch's postulates, and pathogenicity was therefore verified (Ignjatov et al. 2012). Nirmaladevi et al. (2016) reported cultural and morphological characteristics that were further utilized to validate the pathogen's identity in this study. Conidial suspension of FOL was made from a 7-day-old broth culture grown in potato dextrose broth. Each plant in both pots and field trials was inoculated with 10 ml of FOL conidial suspension having 10^7 spores/ml 30 days after transplanting of tomato seedlings.

Collection of root samples and isolation of endophytic *Bacillus*

Root samples of healthy tomato plants were collected from South West Garo hills, West Garo hills, West Jaintia hills, East Jaintia hills and East Khasi hills districts of Meghalaya, India. With slight modifications, the technique described by Zinniel et al. (2002) was used to isolate endophytic *Bacillus*. After washing in running tap water, healthy root surfaces were sterilized in 70% ethanol for 1 min and then sterilized in 2% sodium hypochlorite for 3 min. It was then sterilized in 70% ethanol for 30 s before being rinsed 4–5 times in sterile double distilled water. Roots were cut into tiny pieces and macerated separately with a sterile pestle and mortar in phosphate buffer at pH 7.2. To eliminate additional undesired bacteria, 1.5 ml of the whole extract was suspended in micro centrifuge tubes and given heat shock treatment at 80 °C for 5 min. The tissue extracts were then prepared for serial dilution in sterile saline up to 10^{-5} and 0.1 ml of each dilution of the tissue extract was plated with nutrient agar medium by spread plate method. These plates were then incubated at 28 ± 1 °C for 24 h. Purified bacterial isolates were preserved at 80 °C in 50% glycerol for subsequent investigation.

In vitro antagonistic assay

The antagonistic potential of *Bacillus* isolates against *F. oxysporum* f. sp. *lycopersici* (FOL) was assessed using the dual culture method of Ganesan and Gnanamanickam (1987). Seven-day-old culture of FOL disc of 5 mm was placed at the centre of a fresh PDA plate. Then, 24-h-old each bacterial strain was streaked parallel on both sides of the fungal disc 25 mm away from the disc. Plates without bacterial streak served as control. Three replications were maintained. The plates were kept for incubation at 28 ± 1 °C for 7 days. After control plates reached 90 mm diameter, the growth measurement of the pathogen was recorded and per cent mycelium inhibition over control was worked out by using the following formula:

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

where 'C' is the maximum growth of the fungal mycelium under control condition and 'T' is the fungal mycelium growth in treatment.

Molecular identification of *Bacillus* isolates

Molecular identification was carried out only for those endophytic *Bacillus* isolates that were found most effective in dual culture method. Himedia Bacterial Genomic DNA Purification Kit (MB505-50PR) was used to extract the genomic DNA from selected *Bacillus* isolates. 16S rRNA intervening sequence-specific BCF1 (CGGGAGGCAGCAGTAGGGAAT) and BCR2 (CTCCCCAGGCGGAG TGCTTAAT) primers were used to obtain an amplicon size of 546 bp to confirm strains as *Bacillus* sp. (Cano et al. 1994). PCRs were carried out in 20 µl reaction mixture containing 5 µl of template DNA samples, 2 µl 10X buffer, 1.6 dNTP mixture, 0.5 µl $MgCl_2$, 1 µl forward primer, 1 µl reverse primer, 0.1 µl Taq DNA polymerase and 8.8 µl nuclease free water were amplified on Gradient Master Cycler 5331 using the PCR conditions 1 min at 94 °C, 1 min at 58 °C and 1 min at 72 °C. The overall number of cycles was 40, with the last extension lasting 10 min at 72 °C. 5 µl of amplified 16s rRNA gene product was separated on a 1% (w/v) agarose gel electrophoresis at 50 V for 45 min and visualized and images were captured using gel documentation system (BioRad, CA, USA). The purified PCR products of microbial gene fragments were sent to Agrigenome Labs Pvt. Ltd. in Hyderabad, India, for sequencing. BLAST searches (http://blast.ncbi.nlm.nih.gov/Blast.cgi) were used to compare the sequences to the NCBI database. The sequences were aligned with Clustal W, and a phylogenetic tree was constructed from the evolutionary distances by the neighbour-joining method with the software MEGA7 (Nikunjkumar 2012).

Detection of antifungal antibiotic synthesis genes from selected *Bacillus* endophytes

The presence of antifungal biosynthesis genes for the *Bacillus* isolates found effective in dual culture that specifies the production of antibiotics, viz. iturin A, surfactin and bacillomycin D were screened by using gene-specific primers in PCR-based method. The PCR amplification of iturin A gene (647 bp) [primer ITUD1F (F-5′GATGCG ATCTCCTTGGATGT3′) and ITUD1R (R-5′ATCGTC ATGTGCTGCTTGAG3′], surfactin gene (441 bp) [primers SUR3F (F-5′ACAGTATGGAGGCATGGTC3′) and SUR3R (R-5′TTCCGCCACTTTTTCAGTTT3′)] and bacillomycin D gene (875 bp) [primer BACC1F (F-5′GAA GGACACGGCAGAGAGAGTC3′) and BACC1R (R-5′CGCTGATGACTGTTCATGCT3′)] (Ramarathnam 2007) were carried out in a 20 µl reaction volume containing 5 µl template DNA, 2 µl 10X buffer, 0.5 µl $MgCl_2$, 1.6 µl dNTPs, 1 µl forward Primers, 1 µl reverse primer, 0.1 µl Taq DNA Polymerase and 8.8 µl Nuclease free water. Each amplification product was separated on a 1% (w/v) agarose gel electrophoresis. Gels images were captured using gel documentation system (BioRad, CA, USA). Following PCR conditioning were used to amplify the genes: iturin A gene—initial denaturation at 94 °C for

3 min, 40 cycles consisting of denaturation of 94 °C for 1 min, annealing for 1 min at 60 °C, 72 °C for 1 min off primer extension and final extension 72 °C for 10 min; surfactin—Initial denaturation at 94 °C for 3 min, 40 cycles consisting of 94 °C for 1 min, 57 °C for 1 min, 72 °C for 1 min and final extension 72 °C for 10 min; and bacillomycin D—total of 35 cycles consisting initial denaturation at 94 °C for 3 min, denaturation at 94 °C for 1 min, 60 °C for 30 s, 72 °C for 1 min and final extension 72 °C for 6 min.

Screening for hydrolytic enzyme production

Screening of the selected potential *Bacillus* endophytes for production of hydrolytic enzymes like alpha amylase, cellulase, lipase, protease and pectinase were done by the methods described by Cappuccino and Sherman (1992). For cellulase production, *Bacillus* isolates were spot inoculated on congo red cellulase agar plates at 28 ± 2 °C for 48 h. The presence of clear zones around the bacterial colonies was taken as positive. Tributyrin agar plates were streaked with *Bacillus* isolates and kept at 28 °C for 24–48 h and the appearance of clear zone around the bacterial growth was taken as positive result for lipase production. For protease and pectinase production, one loopful of the bacterial cell suspension was streaked on skim milk agar and pectin agar plate (Mohandas et al. 2018), respectively. After 48 h incubation at 28 °C, appearance of clear zone around the bacterial streak was taken as positive for protease production. For pectinase production, Gram's iodine solution was poured onto pectin agar after 48 h of incubation, and the zone of clearing was examined against a dark blue background.

Screening for antimicrobial secondary metabolites production

Antimicrobial secondary metabolite production such as HCN and siderophore production was screened for the effective *Bacillus* isolates. The test endophytic *Bacillus* isolates were screened for hydrogen cyanide production, following the method described by Lorck (1948). The appearance of orange or red colour on Whatman number 1 filter paper indicated the production of hydrogen cyanide. For siderophore production, Universal Chrome Azurol S (CAS) agar medium as described by Schwyn and Neilands (1987) was used to check siderophore producing ability of *Bacillus* isolates. After incubation for 5–7 days at 28 °C, the presence of an orange zone surrounding the bacterial growth indicated a positive outcome.

Screening for plant growth promotion (PGP) activity

The growth-promoting activities of test *Bacillus* isolates were assessed by following the standard procedure for determining the phosphate solubilization on Pikovskaya's agar medium (Pikovskaya 1948), zinc solubilization on Tris-minimal agar medium supplemented with D-glucose and different insoluble zinc compounds, viz. zinc sulphate ($ZnSO_4$), zinc oxide (ZnO), zinc chloride ($ZnCl_2$) and zinc carbonate ($ZnCO_3$) (Sharma et al. 2011), potassium solubilization on Aleksandrov agar medium (Aleksandrov et al. 1967) and ammonia production (Cappuccino and Sherman 1992). On each individual test media, colour changes and a noticeable halo zone encircling the bacterial colony validated the results of positive plant growth-promoting (PGP) qualities.

Isolation and identification of arbuscular mycorrhizal spores

Wet sieving and decanting method of Gerdemann and Nicolson (1963) was followed to isolates arbuscular mycorrhizal spores from rhizospheric soils of tomato grown in Meghalaya where 50 g rhizosphere soil mixed in one litre water was passed through sieves arranged in a descending order from 500 µm, 212 µm, 106 µm, 53 µm to 38 µm sizes. Spores collected from the sieves were stored in vials containing 2 ml sterile water at 4 °C. The dominant AMF spores were identified using morphological descriptions from the International Culture Collection of Arbuscular Mycorrhizal Fungi (INVAM) website (http://www.invam.caf.wvu.edu), Schenck and Perez (1990) and the AMF phylogeny (www.amf-phylogeny.com). The spores were also sent to National Centre of Fungal Taxonomy (NCFT), New Delhi, for AMF species identification. AMF spores were purified using a technique outlined in INVAM (http://invam.caf.wvu.edu) for single species isolation.

Experimental design

Two AMF species dominating the rhizosphere soils of tomato and 2 endophytic *Bacillus* strains having good PGP and antagonistic activities were evaluated against FOL under pot and field conditions. All the possible treatment combinations were made among the 4 bioinoculants. One negative control (without pathogen), positive control (only pathogen inoculated) and a check fungicide carbendazim were also taken. The pot experiment was conducted at the CPGS-AS campus, CAU, Umiam, Meghalaya, whereas the field experiment was conducted in the farmer's field located at Umiet village, Umroi, Ri-Bhoi District, Meghalaya, which was situated between 91°57′07.2″E longitude and 25°42′33.4″N latitude. A total of 18 treatments were allocated with 3 replications each for both pot and field experiments. 'Rocky' tomato variety was used for the experiments. The plot size of 2 × 2 m^2 with a spacing of 50 × 50 cm was maintained for the field experiment set in a randomized block design (RBD).

The pot experiment was conducted by completely randomized block design (CRD). The crop was sown in the 1st week of January and transplanted on first week of February 2020 in both the field and pot trials.

Sterilization of the soil and pot filling

For both the pot and field trials, tomato seeds variety 'Rocky' were sown in plastic trays filled with sterilized sand: soil mixture (1:2 v/v) and grown for 30 days. Soil and sand in the ratio 3:1 were sterilized in autoclave 3 times at 15lbs/inch^2 for one hour and filled in pots of 30 cm diameter for pot experiment.

Preparation and inoculation of bioinoculants

AMF inocula were mass multiplied using the method of Sharma (2016). 2.5 kg soil mixture containing soil, sand and farm yard manure (FYM) at the ratio of 1:1:1 filled in polypropylene bags was sterilized thrice at 15lbs/inch^2 for one hour. AMF inocula as soil, sand and chopped roots were put in upper 3–5 cm layer of plastic pots (3 kg soil capacity) containing sterilized sand: soil: FYM mixture. Following that, surface sterilized maize (*Zea mays*) seeds were sown in these pots. For 3 to 4 months, the plant was allowed to grow in the containers. The maize roots were chopped and mixed with the potting mixture which is used as an inoculum. The mycorrhizal inocula were separately placed below the seedling by the layering method as described by Menge et al. (1977) @ 200 g soil inoculum (5–6 AM spores/ g soil) per pot and per plant (Kuppusamy and Kumutha, 2012) by spreading the inoculum as a layer at a depth of 3–5 cm in the planting holes of seedlings during the time of transplanting of 30-day-old tomato seedlings.

The two endophytic *Bacillus* strains were cultured separately in Luria broth and then incubated in a rotary shaker of 150 rpm and 28 ± 1 °C. The bacterial suspensions were adjusted to 1×10^8 CFU/ml after 48 h of incubation. 10 ml/plant (1×10^8 CFU/ml) of separate *Bacillus* suspensions and microbial consortium (MC) was used for root dip treatment for 30 min and soil treatment during transplanting. The treatments also included root dip treatment of tomato seedlings with carbendazim @ 0.2% for 10 min before transplanting. Seedlings without any treatment served as negative controls and only pathogen inoculated seedlings served as positive control.

Observations

Disease severity

Disease incidence was measured after 60 and 80 DAT. The 0–4 scale described by Song et al. (2004) was used to record the disease severity. The severity of the disease was graded on a scale of 0 to 4 as given below:

Scale	Intensity
0	: no infection
1	: A slight infection, which is about 25% of full scale, one or two leaves become yellow
2	: Moderate infection, two or three leaves become yellow, 50% of the leaves become wilted
3	: Extensive infection, all plant leaves became yellow, 75% of the leaves become wilted, and growth is inhibited
4	: Complete infection, the whole plant leaves become yellow,100% of the leaves become wilted, and the plants die

Percentage of disease severity was calculated using the formula given by Song et al. (2004):

$$= \frac{\sum \text{Scale} \times \text{Number of plants}}{\text{Highest scale} \times \text{Total number of plants}} \times 100.$$

Disease reduction over positive control

It was calculated by the following formula:

$$\%\ \text{disease reduction} = \frac{C - T}{C} \times 100$$

where $C = \%$ disease severity in positive control and $T = \%$ disease severity in treated plants.

Plant parameters

During the experiment, growth parameters such as number of leaves, plant height (cm) at 30 and 60 Days after transplanting (DAT), root fresh weight (g), shoot fresh weight (g), number of fruits per plant and yield of tomato per plant (kg) were recorded.

Statistical analysis

Dual culture assay was designed in completely randomized design (CRD) with 3 replications for each treatment, and the data were analysed using one-way analysis of variance (ANOVA). The field and pot experiments data were statistically analysed by one-way analysis of variance (ANOVA). Disease severity and disease reduction (%) over positive control in field experiment were transformed using arc sine transformation. If there are significant differences among treatments, it was compared by using Duncan's multiple range test (DMRT) at $p = 0.05$ significance level.

Results

Isolation of *Fusarium* wilt pathogen

Fusarium oxysporum f. sp. *lycopersici* (FOL) was isolated from tomato plants showing *Fusarium* wilt symptoms indicated by vein clearing, yellowing of lower leaves followed by drooping of leaves and young stems along with drying of lower leaves or whole plants and vascular discolouration. The growth of the FOL on PDA medium was fluffy white with purple to violet pigmentation. The microcondia were aseptate, abundant with ellipsoid- to oval-shaped and around 2.4–13.8 × 2.2- 4.1 µm in size while macrocondia were sparse with 3–5 septate, fusiform-shaped and pedicellate base with 16.23–40.5X 3.7-6.4 µm size range.

Pathogenicity test

When the isolated FOL from the diseased plant samples was inoculated on the healthy tomato seedlings by root dip method, *Fusarium* wilt symptoms were observed 7 days after inoculation. On reisolation from artificially inoculated tomato plants, the same fungus was found to associate, and thus, the pathogen was identified as *F. oxysporum* f. sp. *lycopersici* (FOL) by comparing with the culture from repository of CPGS-AS, Umiam, Megalaya, and thus, the pathogenicity of FOL was proved.

Isolation and in vitro antagonistic assay of endophytic *Bacillus* strains

A total of 17 endophytic *Bacillus* isolates were isolated from 5 districts of Meghalaya, India, viz. South West Garo hills, West Garo hills, West Jaintia hills, East Jaintia hills and East Khasi hills districts. By using dual culture method, the 17 isolates were tested for in vitro antagonistic activity against the FOL pathogen. The result showed that isolate ERBS51 had the maximum inhibition (58.43%) of the mycelial growth of the pathogen followed by ERBS10 with 55.68% inhibition (Table 1, Fig. 1). Based on this assay of preliminary screening, isolates ERBS51 and ERBS10 were taken up for further studies.

Table 1 In vitro antagonistic assay of endophytic *Bacillus* isolates against *Fusarium oxysporum* f. sp. *lycopersici*

Sl. no.	*Bacillus* isolates	Mean growth (cm)	Inhibition (%)
1	ERBS10	3.77 ± 0.03	55.68 ± 0.40
2	ERBS17	6.60 ± 0.03	21.96 ± 0.40
3	ERBS48	6.93 ± 0.03	18.43 ± 0.40
4	ERBS51	3.53 ± 0.08	58.43 ± 1.02
5	ERBS59	6.89 ± 0.15	18.82 ± 1.80
6	ERBS67	6.96 ± 0.04	18.03 ± 0.40
7	ERBS77	7.03 ± 0.01	17.25 ± 0.36
8	ERBS78	6.83 ± 0.14	19.60 ± 1.44
9	ERBS91	6.87 ± 0.14	19.21 ± 1.71
10	ERBS93	6.83 ± 0.05	20.00 ± 0.69
11	ERBS97	7.56 ± 0.14	10.97 ± 1.71
12	ERBS103	6.73 ± 0.08	20.78 ± 1.05
13	ERBS106	6.72 ± 0.00	21.17 ± 0.00
14	ERBS110	6.76 ± 0.06	20.39 ± 0.80
15	ERBS111	6.65 ± 0.17	21.56 ± 2.09
16	ERBS112	6.88 ± 0.15	18.82 ± 1.81
17	ERBS122	6.87 ± 0.06	19.21 ± 0.80
18	Control	8.50 ± 0.05	0.00 ± 0.00
	SEM	0.09	1.14
	CD (0.05)	0.28	3.29

Values are the means (± standard error) of three replicates

Molecular identification of *Bacillus* isolates

Molecular identification of *Bacillus* isolates ERBS10 and ERBS51 was done by polymerase chain reaction (PCR) using *Bacillus* genus-specific primers BCF1 and BCR2 for confirmation of the isolates as *Bacillus* group. The primers amplified approximately 546-bp-size region of 16s rRNA intervening sequence, revealing that these isolates were pertaining to *Bacillus* group (Fig. 2). The sequences obtained from the amplified PCR products that were outsourced for sequencing were compared with the GeneBank nucleotide database of NCBI using BLAST (Basic Local Alignment Search Tool). On the basis of 16S rRNA gene sequencing and phylogenetic analysis, the isolates ERBS10 and ERBS51 were identified as *Bacillus* sp. and *Bacillus velezensis*, respectively (Fig. 3). The sequences were submitted to NCBI, and accession numbers MN996896 and MN996898 were assigned for ERBS10 and ERBS51, respectively.

Detection of antifungal antibiotic synthesis genes from selected *Bacillus* endophytes

The result of PCR amplification of antifungal biosynthesis genes (iturin A, surfactin and bacillomycin D) showed that both *B. velezensis* strain ERBS51 and *Bacillus* sp. strain ERBS10 were positive for all the three genes by showing amplification of the genes at approximately 647-bp, 441-bp and 875-bp size specifying the presence of iturin A, surfactin and bacillomycin D biosynthesis genes, respectively (Fig. 4).

Screening for hydrolytic enzyme and antimicrobial secondary metabolites production

Data conferred in Table 2 showed the results of hydrolytic enzyme and antimicrobial secondary metabolites production of *B. velezensis* strain ERBS51 and *Bacillus* sp. strain ERBS10. Both the test *Bacillus* strains were positive for alpha amylase, cellulase, protease production, whereas *Bacillus* sp. strain ERBS10 was negative for lipase production, but *B. velezensis* strain ERBS51

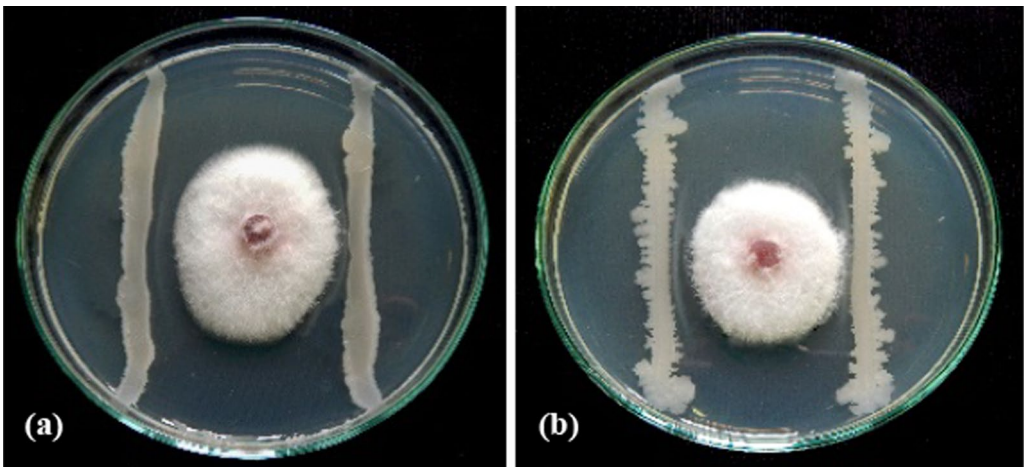

Fig. 1 Dual culture assay of *Bacillus* isolates against *F. oxysporum* f. sp. *lycopersici:* **a** ERBS10 and **b** ERBS51

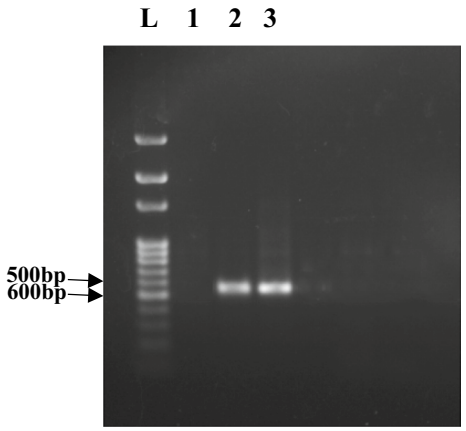

Fig. 2 Amplification of 16s rRNA region of the *Bacillus* isolates using *Bacillus* species-specific primers BCF1 and BCR2; Lane L: 100-bp ladder (Himedia MBT049); Lane 1–3: 1 = blank, 2 = ERBS10, 3 = ERBS51

could produce lipase indicated by formation of halo around the colony. Both the strains were able to produce siderophore; however, both the strains could not produce HCN.

Screening for plant growth promotion (PGP) activity

B. velezensis strain ERBS51 showed a positive result for phosphate solubilization, ammonia production, $ZnCO_3$ and ZnCl solubilization, whereas *Bacillus* sp. strain ERBS10 was found positive for ammonia production and $ZnCO_3$ solubilization (Table 2). Both the test strains were unable to solubilize potassium, $ZnSO_4$ and ZnO.

Isolation and identification of arbuscular mycorrhizal spores

Two dominating AMF species found in rhizospheric soils of tomato grown in Meghalaya were identified as *Funneliformis mosseae* and *Glomus fasciculatum. F. mosseae* had spore diameter ranging between 190–260 µm with brownish yellow, reddish black, sub globose spores having two layers of cell wall with funnel-shaped hyphae attached to the cell wall. *G. fasciculatum* had 130–150 µm spore diameter with dark brown and globose spores having one cell wall layer with no hyphae.

Effect of AMF and endophytic *Bacillus* on disease severity of *Fusarium* wilt of tomato under pot and field conditions

Efficacies of the 2 AMF species (*F. mosseae* and *G. fasciculatum*) and endophytic *Bacillus* (*B. velezensis* strain ERBS51 and *Bacillus* sp. strain ERBS10) alone and in combination along with a check fungicide, carbendazim against FOL under pot and field conditions are given in Tables 3 and 4. Both the AMF and endophytic *Bacillus* were effective in reducing the disease compared to positive control in both the field and pot conditions at both 60 and 80 DAT (Days after transplanting) and their percentage disease reduction in single and combined application in pot and field trials was between 22.26–77.44 and 34.10–66.74% at 80 DAT, respectively. Among all the bioinoculant treatments under pot conditions, the combined application of all the 4 bioinoculants (*F. mosseae* + *G. fasciculatum* + *B. velezensis* + *Bacillus* sp.) was most effective in reducing the disease severity by 77.44% followed by *F. mosseae* + *B. velezensis* + *Bacillus* sp. (66.67%)- and *B. velezensis* + *Bacillus* sp. (66.67%)-treated plants at 80 DAT. Under field conditions, *F. mosseae* + *G.*

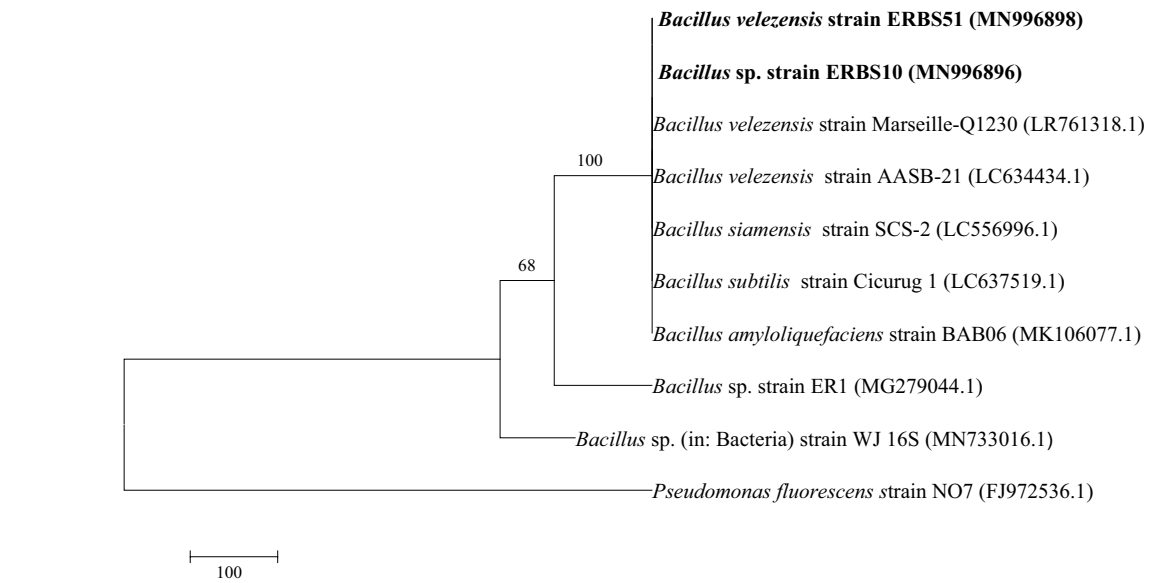

Fig. 3 Neighbour-joining phylogenetic tree based on partial 16S rRNA gene sequences highlighting the position of *Bacillus velezensis* strain ERBS51 and *Bacillus* sp. strain ERBS10 along with reference *Bacillus* strains retrieved from genbank. The numbers at the branches are bootstrap confidence percentages from 1000 bootstrapped trees. *Pseudomonas fluorescens* strain NO7 (FJ972536.1) was used as the out-group

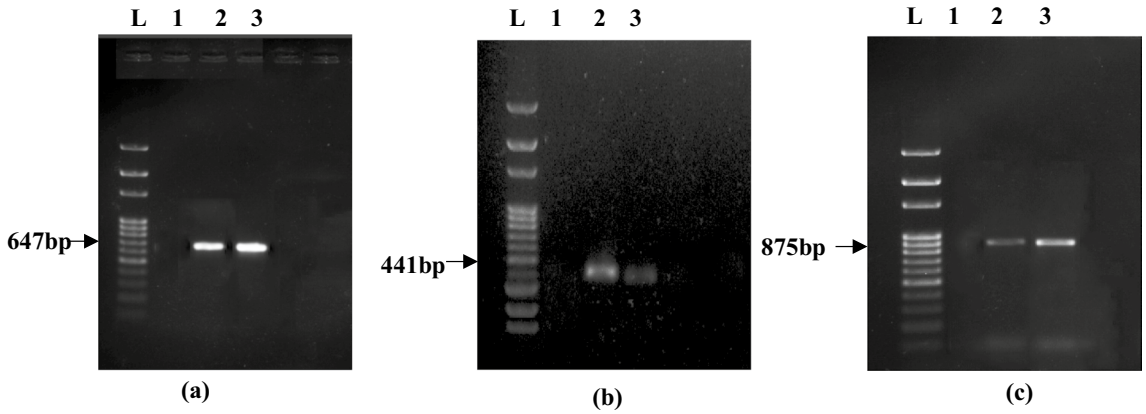

Fig. 4 Amplification of antibiotic synthesis genes for iturin A (**a**), surfactin (**b**) and bacillomycin D (**c**) of the two *Bacillus* strains; Lane L: 100-bp ladder (Himedia MBT049); Lane1-3: 1 = blank, 2 = ERBS10, 3 = ERBS51

fasciculatum + *B. velezensis* + *Bacillus* sp. recorded maximum disease reduction of 66.74%, followed by *F. mosseae* + *B. velezensis* with 60.83% disease reduction which were at par with each other. Among all the treatments, carbendazim as check fungicide recorded the lowest disease severity (8.4 and 10.92%) and highest disease reduction over positive control (88.9 and 71.18%) under pot and field conditions at 80 DAT, respectively. Under pot trial, *Fusarium* wilt incidence was absent in negative control, and in case of field conditions, 2.05% disease severity was recorded. Significant differences were observed in disease severity and disease reduction in all the treatments at 0.05 level of significance in field experiment. Combined application of AMF and *Bacillus* as a consortium gave better disease reduction than applying either AMF or *Bacillus* alone as an individual treatment.

Effect of AMF and endophytic *Bacillus* on growth and yield of tomato under pot and field conditions

Plant height

Data on plant height of 5 randomly selected plants for each treatment under pot and field conditions are

Table 2 Antimicrobial and plant growth promotion activity of the two test *Bacillus* strains

Tests	*Bacillus* sp. strain ERBS10	*Bacillus velezensis* strain ERBS51
Iturin A	+	+
Surfactin	+	+
Bacillomycin D	+	+
Lipase production	−	+
Protease production	+	+
Cellulase production	+	+
Pectinase production	+	+
Alpha-amylase production	+	+
Siderophore production	+	+
HCN production	−	−
Phosphate solubilization	−	+
Ammonia production	+	+
Potassium solubilization	−	−
$ZnCO_3$ solubilization	+	+
$ZnCl_2$ solubilization	−	+
$ZnSO_4$ solubilization	−	−
ZnO solubilization	−	−

presented in Tables 5 and 6. Combined application of *B. velezensis* + *Bacillus* sp. gave the highest average plant height (39 cm) at 30 DAT but at 60 DAT, *F. mosseae* + *G. fasciculatum* + *B.velezensis* + *Bacillus* sp. recorded the highest plant height of 50.36 cm, followed by *F. mosseae* + *G. fasciculatum* (45.44 cm) under pot conditions. In field experiment at initial 30 days after transplanting, *Bacillus* sp. + *F. mosseae* + *G. fasciculatum* showed the maximum plant height of 23.53 cm, but at 60 DAT, *F. mosseae* + *G. fasciculatum* + *B.velezensis* + *Bacillus* sp recorded the highest plant height of 49.41 cm, followed by *F. mosseae* + *B.velezensis* + *Bacillus* sp. (47.52 cm). The lowest height of tomato was recorded in only pathogen inoculated positive control in both the pot and field condtions. Plant height at 30 DAT was non-significant in both the pot and field experiments, but was significant at 60 DAT under both the conditions.

Number of leaves/plant

The average number of leaves per plant was the maximum in *F. mosseae* + *B.velezensis* + *Bacillus* sp.-treated plants in both pot (25.67) and field (21.53) conditions followed by *F. mosseae* + *G. fasciculatum* + *B.velezensis* + *Bacillus* sp (25.33 leaves/plant) in pot experiment and *B.velezensis* + *Bacillus* sp (20.33 leaves/plant) in case

Table 3 Effect of AMF and endophytic *Bacillus* on *Fusarium* wilt of tomato under pot conditions

Treatment details	60 DAT		80 DAT	
	Disease severity (%)	Disease reduction (%) over positive control	Disease severity (%)	Disease reduction (%) over positive control
T_1-*Funneliformis mosseae* (FM)	16.7	49.39	58.3	22.26
T_2-*Glomus fasciculatum* (GF)	25	24.24	58.3	22.26
T_3-*Bacillus velezensis* (BV)	8.4	74.54	41.67	44.45
T_4-*Bacillus* sp. (Bs)	25	24.24	50	33.34
T_5-*F. mosseae* + *B. velezensis* (FM + BV)	0	100	33.4	55.44
T_6- *F. mosseae* + *Bacillus* sp. (FM + Bs)	8.4	74.74	50	33.34
T_7- *G. fasciculatum* + *B. velezensis* (GF + BV)	16.7	49.39	50	33.34
T_8- *G. fasciculatum* + *Bacillus* sp. (GF + Bs)	8.4	74.54	41.67	44.45
T_9- *F. mosseae* + *B. velezensis* + *Bacillus* sp. (FM + BV + Bs)	0	100	25	66.67
T_{10}- *G. fasciculatum* + *B.velezensis* + *Bacillus* sp. (GF + BV + Bs)	16	51.51	41.67	44.45
T_{11}-*B. velezensis* + *F. mosseae* + *G. fasciculatum* (BV + FM + GF)	16	51.51	41.67	44.45
T_{12}-*Bacillus sp.* + *F. mosseae* + *G. fasciculatum* (Bs + FM + GF)	25	24.24	58.3	22.26
T_{13}- *F. mosseae* + *G. fasciculatum* (FM + GF)	16	51.51	41.67	44.45
T_{14}- *B. velezensis* + *Bacillus* sp. (BV + Bs)	0	100	25	66.67
T_{15}- *F. mosseae* + *G. fasciculatum* + *B. velezensis* + *Bacillus* sp. (FM + GF + BV + Bs)	0	100	16.7	77.44
T_{16}-Negative control (without pathogen) (NC)	0	100	0	100
T_{17}-Positive control (only pathogen inoculated) (PC)	33.00	0	75	0
T_{18}-Carbendazim (Car.)	0.00	100	8.4	88.9

DAT days after transplanting

Table 4 Effect of AMF and endophytic *Bacillus* on *Fusarium* wilt of tomato under field conditions

Treatment details	60 DAT		80 DAT	
	Disease severity (%)	Disease reduction (%) over positive control	Disease severity (%)	Disease reduction (%) over positive control
T_1- FM	15.10 ± 1.38(22.82)^{bc}	39.52 ± 0.85(38.95)^{ef}	23.96 ± 2.75(29.23)^{b}	34.10 ± 12.83(34.65)^{b}
T_2- GF	16.66 ± 2.27(23.10)^{b}	33.85 ± 3.17(35.54)^{f}	21.35 ± 1.88(27.49)^{bc}	42.81 ± 3.62(40.84)^{b}
T_3- BV	7.80 ± 3.93(13.44)^{cdef}	71.50 ± 14.28(62.79)^{abcd}	18.21 ± 3.77(24.10)^{bc}	50.15 ± 11.70(45.23)^{b}
T_4- Bs	11.95 ± 1.40(20.17)^{bcd}	51.15 ± 7.68(45.69)^{def}	23.96 ± 1.88(29.27)^{b}	34.86 ± 8.66(35.86)^{b}
T_5- FM + BV	4.13 ± 2.07(9.73)^{def}	84.87 ± 7.57(70.86)^{abc}	14.03 ± 4.72(21.25)^{bc}	60.83 ± 14.48(52.25)^{ab}
T_6- FM + Bs	9.33 ± 1.82(17.62)^{bcde}	61.70 ± 8.13(52.03)^{cdef}	19.81 ± 3.64(26.26)^{bc}	47.77 ± 6.60(43.67)^{b}
T_7- GF + BV	4.69 ± 3.93(9.44)^{def}	82.62 ± 13.72(70.49)^{abc}	17.68 ± 4.29(24.48)^{bc}	51.47 ± 12.98(46.08)^{b}
T_8- GF + Bs	9.85 ± 2.29(18.07)^{bcde}	61.49 ± 5.89(51.73)^{cdef}	20.31 ± 1.80(26.73)^{bc}	45.00 ± 6.69(42.09)^{b}
T_9- FM + BV + Bs	3.60 ± 1.86(9.05)^{ef}	84.05 ± 8.84(70.44)^{abc}	14.55 ± 5.00(21.62)^{bc}	59.17 ± 15.72(51.26)^{ab}
T_{10}- GF + BV + Bs	5.70 ± 2.88(11.45)^{def}	76.16 ± 12.02(65.31)^{abcd}	19.27 ± 3.76(25.86)^{bc}	46.02 ± 15.66(42.06)^{b}
T_{11}- BV + FM + GF	4.67 ± 2.38(10.33)^{def}	79.85 ± 11.13(67.72)^{abcd}	14.56 ± 2.78(22.23)^{bc}	59.55 ± 10.75(50.76)^{ab}
T_{12}- Bs + FM + GF	3.12 ± 2.38(7.94)^{ef}	85.24 ± 11.94(72.35)^{abc}	18.08 ± 5.91(24.59)^{bc}	51.64 ± 14.25(46.07)^{b}
T_{13}- FM + GF	8.85 ± 4.45(14.37)^{bcde}	67.65 ± 16.20(60.36)^{bcde}	20.29 ± 5.51(26.26)^{bc}	47.76 ± 11.30(43.76)^{b}
T_{14}- BV + Bs	3.12 ± 2.39(7.94)^{ef}	85.22 ± 11.96(72.33)^{abc}	17.16 ± 4.16(24.12)^{bc}	55.46 ± 8.10(48.22)^{b}
T_{15}- FM + GF + BV + Bs	2.05 ± 1.35(6.67)^{ef}	91.99 ± 4.73(76.47)^{abc}	11.48 ± 4.94(18.78)^{bcd}	66.74 ± 16.78(56.23)^{ab}
T_{16}- NC	0.00 ± 0.00(0.35)^{f}	100.00 ± 0.00(89.42)^{a}	2.05 ± 1.35(6.67)^{d}	94.56 ± 3.28(78.99)^{a}
T_{17}- PC	25.00 ± 2.39(29.94)^{a}	0.00 ± 0.00(0.57)^{g}	37.49 ± 3.12(37.72)^{a}	0.00 ± 0.00(0.46)^{c}
T_{18}- Car.	1.57 ± 0.91(5.97)^{ef}	94.31 ± 3.24(78.60)^{ab}	10.92 ± 1.80(19.18)^{cd}	71.18 ± 2.95(57.59)^{ab}
SEM	4.19	9.00	3.05	6.11
CD (0.05%)	11.78	25.48	8.75	17.61

Data in parentheses are arc sine transformed values

Values with different letter(s) within a column are significant at p< 0.05 by Duncan's multiple range test (DMRT), Values are the means (±standard error) of three replicates

of field experiment (Tables 4 and 5). There were non-significant differences in the number of leaves per plant under pot conditions.

Number of fruits/plant

The highest average number of fruits per plant in pot condition was recorded in *F. mosseae* + *G. fasciculatum* + *B. velezensis* + *Bacillus* sp. (12 nos.) and *B.velezensis* + *F. mosseae* + *G. fasciculatum* (12 nos.), whereas in case of field condition *G. fasciculatum* + *B.velezensis* (10.2 nos.) and *B.velezensis* + *F. mosseae* + *G. fasciculatum* (10 nos.) had the highest number of fruits per plant (Tables 4 and 5).

Root fresh weight (g)

Average root fresh weight was the highest in *F. mosseae* + *G. fasciculatum* + *B.velezensis* + *Bacillus* sp. under both pot and field conditions with 29.76 and 31.35 g, respectively, followed by *F. mosseae* + *G. fasciculatum* (27.53 g) in pot and *F. mosseae* + *B.velezensis* + *Bacillus* sp.(29.67 g) in field conditions (Tables 4 and 5). They were significant in both pot and field conditions.

Shoot fresh weight (g)

Average shoot fresh weights of 5 randomly selected plants of both pot and field conditions were given in Tables 4 and 5. The data showed that *F. mosseae* + *B. velezensis* + *Bacillus* sp. gave the maximum shoot fresh weight of 45.57 g, followed by *G. fasciculatum* + *B. velezensis* (45.35 g) under pot conditions, whereas under field conditions *F. mosseae* + *G. fasciculatum* + *B.velezensis* + *Bacillus* sp. had the highest shoot fresh weight of 45.3 g, followed by *F. mosseae* + *B.velezensis* + *Bacillus* sp. (44.7 g).

Yield per plant (kg)

In pot experiment, the highest yield was observed in *F. mosseae* + *G. fasciculatum* + *B.velezensis* + *Bacillus* sp. (0.610 kg/plant), followed by *F. mosseae* + *B.velezensis* (0.601 kg/plant), whereas in case of field experiment *F. mosseae* + *B.velezensis* + *Bacillus* sp. (0.709 kg /plant) gave the maximum yield, followed by *F. mosseae* + *G. fasciculatum* + *B.velezensis* + *Bacillus* sp. (0.701 kg/plant) (Tables 4 and 5). The lowest yield of tomato was recorded in positive control in both the conditions. Yield

Table 5 Effect of AMF and endophytic *Bacillus* on growth attributes and yield of tomato under pot conditions

Treatment details	Plant height		No. of leaves/ plant	No. of fruits/ plant	Root fresh weight (g)	Shoot fresh weight (g)	Yield per plant (kg)
	30 DAT	60 DAT					
T_1- FM	36.87 ± 2.81^{a}	39.00 ± 4.04^{bc}	21.00 ± 2.08^{ab}	7.33 ± 0.33^{b}	20.54 ± 3.55^{abcdef}	38.70 ± 2.45^{abcd}	0.461 ± 0.00^{e}
T_2- GF	34.50 ± 1.32^{ab}	38.67 ± 2.60^{bc}	23.33 ± 2.03^{ab}	9.00 ± 0.58^{b}	16.67 ± 1.44^{cdef}	35.42 ± 3.60^{bcde}	0.500 ± 0.01^{d}
T_3- BV	34.67 ± 1.76^{ab}	40.33 ± 2.85^{bc}	20.33 ± 1.86^{abc}	7.00 ± 0.58^{bc}	12.44 ± 1.63^{ef}	39.17 ± 2.63^{abc}	0.449 ± 0.01^{e}
T_4- Bs	36.00 ± 1.73^{ab}	37.33 ± 2.19^{bc}	20.00 ± 1.53^{abc}	7.67 ± 0.67^{b}	14.38 ± 2.92^{def}	36.55 ± 1.27^{bcd}	0.455 ± 0.011^{e}
T_5- FM + BV	33.00 ± 3.79^{ab}	38.33 ± 3.28^{bc}	21.67 ± 3.93^{ab}	10.00 ± 1.15^{ab}	18.20 ± 2.92^{bcdef}	39.26 ± 1.67^{abc}	0.601 ± 0.01^{ab}
T_6- FM + Bs	36.53 ± 1.30^{a}	43.00 ± 1.73^{ab}	20.67 ± 2.19^{ab}	8.00 ± 0.58^{b}	22.33 ± 3.75^{abcde}	30.59 ± 1.33^{de}	0.554 ± 0.01^{c}
T_7- GF + BV	34.97 ± 1.58^{ab}	43.90 ± 5.41^{ab}	23.00 ± 1.73^{ab}	9.67 ± 0.33^{ab}	22.74 ± 2.09^{abcde}	45.35 ± 1.16^{a}	0.509 ± 0.00^{d}
T_8- GF + Bs	34.87 ± 2.95^{ab}	38.33 ± 3.28^{bc}	21.67 ± 2.73^{ab}	8.67 ± 1.45^{b}	25.95 ± 4.11^{abc}	38.90 ± 2.63^{abcd}	0.451 ± 0.00^{e}
T_9- FM + BV + Bs	36.07 ± 2.31^{ab}	43.00 ± 2.65^{ab}	25.67 ± 1.45^{a}	10.00 ± 1.15^{ab}	25.54 ± 2.61^{abc}	45.57 ± 1.96^{a}	0.600 ± 0.01^{ab}
T_{10}- GF + BV + Bs	32.00 ± 1.53^{ab}	37.67 ± 1.67^{bc}	22.00 ± 2.00^{ab}	9.33 ± 0.33^{ab}	21.21 ± 1.86^{abcde}	33.32 ± 1.86^{bcde}	0.536 ± 0.00^{c}
T_{11}- BV + FM + GF	33.67 ± 3.76^{ab}	38.67 ± 3.18^{bc}	23.67 ± 1.45^{ab}	12.00 ± 1.15^{a}	23.37 ± 4.02^{abcd}	36.34 ± 1.32^{bcd}	0.583 ± 0.01^{b}
T_{12}- Bs + FM + GF	33.00 ± 3.46^{ab}	38.33 ± 3.28^{bc}	22.67 ± 0.67^{ab}	9.33 ± 1.33^{ab}	18.76 ± 3.94^{bcdef}	38.43 ± 3.86^{abcd}	0.534 ± 0.01^{c}
T_{13}- FM + GF	32.00 ± 1.15^{ab}	45.44 ± 2.60^{ab}	24.67 ± 1.86^{a}	8.67 ± 0.88^{b}	27.53 ± 2.63^{ab}	40.25 ± 3.00^{ab}	0.592 ± 0.01^{ab}
T_{14}- BV + Bs	39.00 ± 1.73^{a}	37.52 ± 3.02^{bc}	21.33 ± 0.88^{ab}	8.33 ± 0.88^{b}	19.91 ± 1.17^{abcdef}	41.23 ± 1.05^{ab}	0.553 ± 0.01^{c}
T_{15}- FM + GF + BV + Bs	38.00 ± 3.21^{a}	50.36 ± 2.36^{a}	25.33 ± 1.20^{a}	12.00 ± 1.53^{a}	29.76 ± 3.82^{a}	45.24 ± 2.50^{a}	0.610 ± 0.01^{a}
T_{16}- NC	31.67 ± 0.88^{ab}	42.67 ± 1.20^{bc}	17.67 ± 0.88^{bc}	7.00 ± 0.58^{bc}	14.67 ± 4.07^{def}	31.01 ± 2.62^{cde}	0.440 ± 0.01^{e}
T_{17}- PC	28.00 ± 1.15^{b}	32.00 ± 1.15^{bc}	14.33 ± 0.33^{c}	4.33 ± 0.33^{c}	10.66 ± 1.34^{f}	27.40 ± 4.64^{e}	0.263 ± 0.00^{f}
T_{18}- Car.	34.33 ± 2.85^{ab}	41.67 ± 4.06^{bc}	22.00 ± 3.06^{ab}	7.33 ± 0.33^{b}	18.67 ± 4.22^{bcdef}	36.01 ± 2.38^{bcd}	0.463 ± 0.01^{e}
SEM	2.38	2.79	1.96	0.89	3.08	2.52	0.008
CD (0.05%)	6.82 (NS)	7.99	5.63 (NS)	2.54	8.83	7.24	0.024

Values with different letter(s) within a column are significant at $p< 0.05$ by Duncan's multiple range test (DMRT), Values are the means (±standard error) of three replicates

was found significantly different in both pot and field experiments.

Discussion

Fusarium wilt of tomato caused by *F. oxysporum* f. sp. *lycopersici* occurs all over the world. In order to achieve environmental and economic sustainability in crop protection, new control techniques must be developed (Hernández-Aparicio et al. 2021).

Bacillus species could survive endophytically inside plant tissue with tremendous colonization ability and they could also thrive under adverse environments. In the present study, a total of 17 endophytic *Bacillus* species were isolated from tomato roots collected from 5 districts of Meghalaya, India. Out of them, only 2 *Bacillus* isolates, i.e. ERBS51 and ERBS10, were found most effective in inhibiting the mycelial growth of the fungal pathogen FOL by 58.43 and 55.68%, respectively. ERBS51 and ERBS10 were identified as *Bacillus velezensis* and *Bacillus* sp., respectively, based on 16s rRNA gene sequencing. Many authors also reported the inhibition of FOL by *Bacillus* species (Kamali et al. 2019). There are many studies exploring the potential of *Bacillus* species and *Bacillus velezensis* as a biological control agent against a wide range of plant pathogenic fungi (Kim et al. 2021). This success of antagonism by the *Bacillus* isolates maybe contributed to the production of antibiotic, hydrolytic enzyme and secondary metabolite.

In the present study, both *Bacillus* strains were confirmed for the presence of iturin A, surfactin and bacillomycin D biosynthesis genes. The results were supported by earlier findings who reported the production of genes, viz. iturin A, surfactin and bacillomycin D in *Bacillus* spp. (Theatre et al. 2021). Antimicrobial antibiotics helps in formation of biofilm, swarming motility of *Bacillus* and cell damage by creating ion-conducting holes, thereby having strong antimicrobial activities against pathogens (Saxena et al. 2020). Lytic enzymes produced by *Bacillus* spp. have the capacity to degrade fungal cell wall which in term helped to antagonize fungal pathogens (Leelasuphakul et al. 2006). Siderophore producing *Bacillus* spp. played an important role in biocontrol of many soilborne fungal plant pathogens by increasing uptake of iron (Goswami et al. 2016). Obtained result showed that both the *Bacillus* isolates could produce siderophore. But both *Bacillus* strains could not produce HCN.

Different strains of *Bacillus* have been linked with plant growth-promoting (PGP) characteristics (Gamez

Table 6 Effect of AMF and endophytic *Bacillus* on growth attributes and yield of tomato under field conditions

Treatment details	Plant height		No. of leaves/ plant	No. of fruits/ plant	Root fresh weight (g)	Shoot fresh weight (g)	Yield per plant (kg)
	30 DAT	60 DAT					
T_1- FM	20.37 ± 2.43^{ab}	39.83 ± 2.35^{abc}	16.08 ± 1.95^{bcd}	6.78 ± 0.93^{bc}	20.57 ± 3.38^{cde}	39.90 ± 3.49^{ab}	0.609 ± 0.00^{c}
T_2- GF	19.40 ± 2.57^{ab}	40.89 ± 2.00^{abc}	17.83 ± 1.88^{abc}	7.32 ± 0.50^{abc}	25.76 ± 1.91^{abc}	35.23 ± 3.17^{abcde}	0.500 ± 0.01^{f}
T_3- BV	19.57 ± 2.44^{ab}	38.37 ± 2.61^{bc}	16.33 ± 0.65^{abc}	6.00 ± 0.19^{c}	12.33 ± 2.59^{fg}	31.47 ± 1.94^{bcde}	0.494 ± 0.00^{f}
T_4- Bs	20.07 ± 2.38^{ab}	36.89 ± 7.23^{cd}	14.75 ± 3.17^{cd}	7.75 ± 0.90^{abc}	11.43 ± 2.34^{fg}	27.90 ± 4.38^{de}	0.471 ± 0.00^{g}
T_5- FM + BV	21.83 ± 1.30^{ab}	41.55 ± 1.39^{abc}	18.08 ± 1.76^{abc}	7.75 ± 0.55^{abc}	23.55 ± 2.71^{bcd}	33.14 ± 2.39^{bcde}	0.645 ± 0.01^{b}
T_6- FM + Bs	20.45 ± 2.95^{ab}	41.28 ± 2.47^{abc}	19.92 ± 1.16^{abc}	7.18 ± 0.97^{abc}	18.07 ± 1.92^{de}	32.65 ± 2.46^{bcde}	0.535 ± 0.01^{e}
T_7- GF + BV	21.91 ± 1.39^{ab}	38.53 ± 2.55^{bc}	18.58 ± 1.71^{abc}	10.20 ± 1.33^{a}	29.35 ± 1.82^{ab}	39.24 ± 3.53^{abc}	0.680 ± 0.00^{b}
T_8- GF + Bs	20.67 ± 1.92^{ab}	39.60 ± 1.45^{bc}	18.75 ± 1.01^{abc}	8.17 ± 1.48^{abc}	26.53 ± 3.13^{abc}	41.23 ± 2.45^{ab}	0.567 ± 0.01^{d}
T_9- FM + BV + Bs	20.77 ± 2.13^{ab}	47.52 ± 1.31^{ab}	21.53 ± 0.65^{a}	9.87 ± 0.73^{a}	29.67 ± 2.71^{ab}	44.70 ± 3.44^{a}	0.709 ± 0.00^{a}
T_{10}- GF + BV + Bs	22.08 ± 1.94^{ab}	42.17 ± 2.32^{abc}	18.53 ± 1.46^{abc}	7.30 ± 0.72^{abc}	19.43 ± 2.54^{cde}	35.55 ± 2.26^{abcd}	0.554 ± 0.01^{d}
T_{11}- BV + FM + GF	20.72 ± 2.45^{ab}	41.00 ± 2.40^{abc}	17.58 ± 1.21^{abc}	6.78 ± 1.13^{bc}	24.13 ± 2.15^{abcd}	40.25 ± 3.53^{ab}	0.532 ± 0.01^{e}
T_{12}- Bs + FM + GF	23.53 ± 1.64^{a}	45.76 ± 3.89^{abc}	17.00 ± 1.01^{abc}	7.89 ± 0.0^{abc}	26.76 ± 0.57^{abc}	36.97 ± 1.61^{abcd}	0.612 ± 0.01^{c}
T_{13}- FM + GF	18.44 ± 0.98^{ab}	41.17 ± 1.92^{abc}	17.83 ± 0.93^{abc}	8.31 ± 1.07^{abc}	24.10 ± 2.35^{abcd}	40.33 ± 3.74^{ab}	0.594 ± 0.00^{c}
T_{14}- BV + Bs	19.55 ± 1.49^{ab}	39.28 ± 3.71^{bc}	20.33 ± 1.86^{ab}	9.27 ± 0.91^{ab}	17.34 ± 1.88^{de}	33.47 ± 2.19^{bcde}	0.573 ± 0.01^{d}
T_{15}- FM + GF + BV + Bs	21.29 ± 1.70^{ab}	49.41 ± 2.92^{a}	20.17 ± 0.42^{abc}	10.00 ± 1.53^{a}	31.35 ± 0.77^{a}	45.30 ± 1.11^{a}	0.701 ± 0.00^{a}
T_{16}- NC	22.13 ± 1.35^{ab}	43.68 ± 0.97^{abc}	18.83 ± 1.58^{abc}	7.33 ± 0.33^{abc}	11.10 ± 2.46^{fg}	27.47 ± 4.16^{de}	0.471 ± 0.00^{g}
T_{17}- PC	16.80 ± 1.33^{ab}	29.43 ± 2.85^{d}	11.47 ± 0.74^{d}	6.02 ± 0.35^{c}	8.23 ± 1.80^{g}	25.27 ± 2.71^{e}	0.391 ± 0.00^{h}
T_{18}- Car.	15.51 ± 1.67^{b}	39.05 ± 0.64^{bc}	15.11 ± 0.49^{cd}	6.21 ± 0.32^{bc}	14.76 ± 0.14^{efg}	29.47 ± 3.52^{cde}	0.501 ± 0.00^{f}
SEM	1.98	2.93	1.45	0.91	2.21	3.05	0.007
CD (0.05%)	5.70 (NS)	8.43	4.16	2.61	6.36	8.77	0.019

Values with different letter(s) within a column are significant at $p< 0.05$ by Duncan's multiple range test (DMRT), Values are the means (±standard error) of three replicates

et al. 2019). In the present investigation, only *B. velezensis* strain ERBS51 showed the ability to solubilize phosphate which was similar to the finding of Thilagavathi and Prasad (2020) who found that *B. velezensis* and *Bacillus* specie were able to solubilize phosphate and promote plant growth. Both *Bacillus* strains were able to produce ammonia in the present study.

In the present study, *F. mosseae* and *G. fasciculatum* were the dominating AMF species in tomato rhizosphere of Meghalaya. Both the AMF species belonged to Glomeraceae family and the abundance of this family may be due to the ability of some species to colonize previously colonized AMF roots and its capability to acclimatize disturbed environment (House and Bever 2018). The reduction in wilting in AMF inoculated plants may be attributed to fusaric acid production, and they directly reduced the wilt inside vascular tissue. AMF also increases the activity of enzymes that metabolizes phosphate such as alkaline and acid phosphatase and also increases chlorophyll contents of the plant, thereby reducing the damage of *Fusarium* wilt (Hashem et al. 2021).

Through the observations of the present study, it was found that out of all the bioinoculant-treated plants, *F. mosseae* + *G. fasciculatum* + *B.velezensis* + *Bacillus* sp. as a combined application was found most effective in reducing the wilt severity to 77.44 and 66.74%, under both pot and field trials compared to other treatments. Similarly, under pot and field conditions the consortium of *F. mosseae* + *G. fasciculatum* + *B. velezensis* + *Bacillus* sp. was the best in terms of most growth parameters and yield. The result showed that application of AMF and *Bacillus* as an individual treatment were not found much effective compared to combined application as a consortium both under pot and field experiments in enhancing the disease suppression, growth and yield attributes. In both conditions, consortium of *F. mosseae* + *G. fasciculatum* + *B. velezensis* + *Bacillus* sp. showed the best result. Several researchers has also reported the co-inoculation of AMF and *Bacillus* as an effective strategy for reducing the occurrence of diseases like *Fusarium* wilt, root rot, *Fusarium* crown rot in tomato and also enhanced plant growth (Cai et al. 2021). AMF such as *Glomus macrocarpum*, *Rhizophagus intraradices*, *G. austral* and *G. mosseae* could control bacterial and *Fusarium* wilt of tomato, *Fusarium* root and pod rot diseases of peanut (Hashem et al. 2021). Similarly, there were reports of *B.*

subtilis and *B. velezensis* reducing the severity of *Fusarium* wilt in tomato, cucumber and banana (Wu et al. 2020). AMF and endophytic *Bacillus* formed a mutual symbiosis, thereby performing a synergistic role by colonizing the rhizosphere and roots of the tomato plants. They formed a positive biological effect when they were combined together.

Conclusions

The present study demonstrated that *Bacillus velezensis* strain ERBS51 and *Bacillus* sp. strain ERBS10 were potential biocontrol agents with good antimicrobial and PGP attributes. *F. mosseae* + *G. fasciculatum* + *B. velezensis* + *Bacillus* sp. as a combination had a strong *Fusarium* wilt reducing ability along with enhancing yield and some growth traits of tomato under both pot and field conditions compared to other combinations. Therefore, these microbes as a combination can be recommended as biocontrol agents and as a tool in integrated disease management of *Fusarium* wilt of tomato.

Abbreviations

AMF: Arbuscular mycorrhiza fungi; FOL: *Fusarium oxysporum* F. sp. *lycopersici*; CFU/ml: Colony-forming unit per millimetre; DAT: Days after transplanting; RBD: Randomized block design; CRD: Completely randomized block design; PCR: Polymerase chain reaction; dNTPs: Deoxyribonucleotide triphosphate; PGP: Plant growth promotion; FM: *Funneliformis mosseae*; GF: *Glomus fasciculatum*; BV: *Bacillus velezensis*; BS: *Bacillus* Sp.; NC: Negative control; PC: Positive control; Car.: Carbendazim; CFU: Colony-forming unit; DMRT: Duncan's multiple range test.

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Authors' contributions

NOD performed the experiments, analysed the data and prepared the original manuscript. MD and ST contributed to molecular analysis and isolation of *Bacillus* and AMF. RKTD and MH contributed to supervision, editing, conceptualization, analysing and interpretation of the data. All the authors also contributed to reviewing and editing of the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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