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# Enhancing the Biocontrol Potential of Compost and Vermicompost Tea with *Bacillus* spp. Against Bacterial Wilt of Tomato

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The ability to suppress *Ralstonia solanacearum* using compost and vermicompost tea enriched with *Bacillus* spp. was assessed both under *in vitro* and *in vivo* conditions in this study. Tomato is one of the most important vegetables in the world, which are highly susceptible to bacterial wilt disease. Three distinct media were used to evaluate the antagonistic efficacy of 21 *Bacillus* isolates, of which 14 isolates showed an antagonistic effect and three isolates, $B_1$ – *Bacillus megaterium* (B40DhanSat), $B_2$ – *Bacillus cereus* (BD49IslamSat) and $B_3$ – *Bacillus cereus* (EB36KalSat), exhibited the highest inhibition percentage, particularly in vermicompost tea media. Vermicompost and compost tea were enriched with three promising antagonistic isolates and used as treatments on the tomato variety named 'Roton'. Disease incidence and severity [%] measured at 30, 60 and 90 days after transplanting (DAT) showed that $T_4$ (soil + vermicompost tea + $B_2$ 8%), $T_{10}$ (soil + vermicompost tea + $B_1$ + $B_2$ + $B_3$ 7.9%) and $T_{12}$ (Bactroban,7.37%) had the highest disease suppression ability than control treatment at 90 DAT, following the application of total twelve treatments. Data recorded on vegetative, reproductive, as well as biochemical parameters at various growth stages showed the best performance of $T_4$, $T_{10}$ and $T_{12}$ treatments. Thus, it can be concluded that compost and vermicompost tea fortified with *Bacillus* spp. can have a potential suppressive effect on the bacterial wilt pathogen of tomato by reducing mycelial growth. Therefore, farmers may be guided to use eco-friendly organic amendments, ensuring efficient disease management without adverse effects on human health or environmental safety.

Keywords: biocontrol agents, *Ralstonia solanacearum*, tomato wilt, compost and vermicompost extract, antagonistic ability

One of the commonly cultivated important vegetables in the world after the potato is tomato (*Solanum lycopersicum* L.), which is a member of the Solanaceae family (Lal *et al*. 2017). Tomatoes are not only used as a vegetable for culinary purposes, but they are also full of antioxidants, which are beneficial for human health. Tomatoes are now available year-round having increased levels of vitamins A, B, and C as well as calcium and carotene, with approximately 94% water content (Mengesha *et al*. 2017). Despite a gradual, significant growth in total planted area and tomato production over the past few years in the country, productivity is still quite low at 6.46 tons per hectare when compared to the global average of 40.84 tons per hectare (FAOSTAT 2022).

*Ralstonia solanacearum*, the cause of bacterial wilt, is the world's second-largest plant bacterial disease, which causes great damage to the global output (Tessema 2023). Nowadays, bacterial wilt management has been difficult because of the limi-

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tations of efficient strategies, especially when single cropping is practised continuously, and climate change has an impact (Wu *et al*. 2023). Biocontrol of this pathogen alone is relatively ineffective due to its high genetic diversity and ability to adapt to various environmental conditions (Mengesha *et al.* 2017).

Although some bacterial wilt-resistant cultivars have been developed, their resistance is limited to certain soil types, climates, and pathogen strains (Vanitha *et al.* 2009).

Globally, insects, weeds and plant diseases collectively reduce crop productivity by 36% while diseases by themselves have been shown to reduce crop yield by 14% (Agrios 2005). Beneficial organisms are affected by the application of pesticides even if it is applied at their prescribed dose. In order to address these issues, compost and vermicompost extract with biocontrol agents must be used as a safe and effective substitute against the diseases which have been crucial in recent years (Yatoo *et al*. 2021). A variety of cyclic peptides that are active against different microbes are produced by *Bacillus* species, which makes them suitable as effective biocontrol agents (Sivakumar *et al*. 2014).

Vermicompost contains a number of nutrients, including phosphate, nitrate and potassium, which are made available to plants by the presence of helpful bacteria. Plant diseases have been effectively managed over the past 20 years with the use of the liquid form of vermicompost (Yatoo *et al*. 2021). Vermicast is thought to transport nutrients and microorganisms into a liquid solution, called vermicompost tea, making it more useful (Wang *et al*. 2019; Yatoo *et al*. 2021). Compost tea offers several advantages over direct compost by increasing the number of biologically active microorganisms and applying a selection pressure that can be readily enhanced (Yatoo *et al*. 2021). The fortification of compost extract with biological control agents offers a successful substitute method for disease control by inducing resistance and enhancement of soil fertility (Abbasi *et al*. 2002). There is also substantial evidence that compost and vermicompost teas, when applied as soil drenches, can inhibit a variety of soil-borne diseases (Marín *et al*. 2013). Therefore, the study was conducted to ensure the effective management of bacterial wilt disease of tomato in an environmentally friendly method by the use of compost and vermicompost tea fortified with *Bacillus* spp.

## MATERIAL AND METHODS

*Source and Maintenance of the Pathogen*

*Ralstonia solanacearum* pathogen was collected from BINA (Bangladesh Institute of Nuclear Agriculture), which was previously isolated near Bangladesh Agricultural University, Mymensingh. Growing colonies were picked from collected plates and sub-cultured into TZC (2, 3, 5-triphenyl tetrazolium chloride) media and preserved in 20% glycerol for subsequent use.

*Pathogenicity Test*

For the pathogenicity test, a needle was dipped into the suspension of the pathogen inoculum. At the three to four true leaf stage of the healthy tomato plant, the stem was punctured at the axils to make the inoculation with the pathogenic strain (Winstead & Kelman 1952). In a greenhouse, inoculated plants were kept between 26 and 30°C with 12 hours of light and 12 hours of darkness. Plants were left without water for 24 hours before inoculation (Williamsson *et al*. 2002). After the inoculation, examination of the infected plants was performed by critically observing the symptoms such as leaf yellowing, wilting and stunted growth (Ahmed *et al*. 2013).

*Source of Biological Control Agents (BCAs)*

Fourteen isolates of *Bacillus* spp. were collected from the Probiotic and Bio-formulations Laboratory, Department of Plant Pathology, Bangladesh Agricultural University, which were previously isolated following the dilution plate technique of rhizospheric soil by gently uprooting healthy tomato plants and carefully shaking off the loosely attached soil. The soil still adhering tightly to the roots (approximately 1 – 3 mm layer around the root surface) was considered as rhizosphere soil. This soil was collected in sterile bags, homogenised, and used for Bacillus isolation. The molecular identification of these isolates was done previously by DNA extraction and gene sequencing. The sequences were then compared against the NCBI (National Center for Biotechnology Information) database to perform

BLAST analysis and obtain accession numbers (Table 1).

*Source and Maintenance of Compost and Vermicompost*

Commercially available compost and vermicompost were collected from Al-Arafah and Ispahani Agro Limited, respectively, located in Dhaka, Bangladesh, for the preparation of compost and vermicompost tea in this experiment. Until compost tea was prepared, samples were kept in non-airtight containers at room temperature (22 – 25°C) for 24 – 48 hours.

*Preparation of Non-Aerated Compost and Vermicompost tea*

Commercially available compost and vermicompost were used for the preparation of compost and vermicompost tea. Non-aerated compost tea was prepared in a 30 L plastic bucket at a ratio of 1:5 (w/v) using water from agricultural wells and kept at room temperature for 14 days. Compost ferment was filtered using layered muslin cloth (Koné *et al*. 2010). To prepare vermicompost tea, four litres of tap water were combined with one litre of vermicompost in a clean container. The mixture was thoroughly stirred until froth or bubbles appeared, indicating active microbial activity. The suspension was then left undisturbed for 24 – 48 hours to allow fermentation, after which it was strained through a fine mesh to obtain the liquid extract (Edwards *et al.* 2010).

Table 1

Details of the molecular identification of selected biological control agents

| BCAs | Name | Accession no |
|---|---|---|
| SH4E1 | *Bacillus cereus* | PQ637437 |
| SH10E1 | *Bacillus licheniformis* | PQ637438 |
| SH41E2 | *Bacillus cereus* | PQ637439 |
| SH 44E2 | *Bacillus amyloliquefaciens* | PQ637440 |
| SH 44E4 | *Bacillus subtilis* | PQ637441 |
| BDISOB08KhuDu | *Bacillus cereus* | OR921624 |
| LYSX73 | *Bacillus subtilis* | OR673722 |
| BDISOB109KhuBa | *Bacillus subtilis* | OR921632 |
| BDISOB81KhuDu | *Bacillus amyloliquefaciens* | OR921629 |
| BDISOB121KhuBa | *Bacillus aquimaris* | PP328581 |
| EGY-SCJ5 | *Bacillus methylotrophicus* | PP328530 |
| BDISOB41KhuDu | *Bacillus subtilis* | OR921627 |
| B22ChilaBgr | *Bacillus flexus* | OR994300 |
| BD49IslamSat | *Bacillus cereus* | OR921608 |
| B21BurirBgr | *Bacillus aquimaris* | OR921601 |
| B40DhanSat | *Bacillus megaterium* | OR921606 |
| EB28NachSat | *Bacillus cereus* | OR975555 |
| EB29NachSat | *Bacillus cereus* | OR975556 |
| EB36KalSat | *Bacillus cereus* | OR975557 |
| B43TalaSat | *Bacillus flexus* | OR921607 |
| BDISOB221KhuDa | *Halobacillus massiliensis* | OR921645 |

Note: BCAs – biological control agents.

*Non-Erated Compost and Vermicompost Tea Media Preparation*

Non-sterilised compost and vermicompost tea media were prepared according to the method described by Islam *et al*. (2019) with some modifications. Modified lysogeny agar media was prepared by adding 3 mL of either compost or vermicompost tea with 17 mL of lysogeny agar media. A control plate was used with lysogeny agar media by adding only 15% sterile distilled water. 0.1% of NaOH alkaline solution was added to the media to bring their pH down to 6.5.

*Antimicrobial Activity of Antagonistic Bacterial Strains*

Antagonism activity was evaluated according to the protocol of Jiang *et al*. (2015) and Udayashankar *et al*. (2009) with some modifications. 1 L of LB medium was combined with 10 mL of bacterial suspension of *Ralstonia solanacearum* having $1.0 \times 10^7$ CFU/mL, stirred and transferred into plates where a temperature below 50°C and an optical density of 0.3 was maintained. After spot inoculation of each of the 14 bacterial test antagonists (5 µL of $10^8$ CFU/mL) onto the centre of the plates, they were incubated for two days at 28°C. Following incubation, the diameter of the transparent semi-circular halo zone, which indicated the growth inhibition by the antagonistic bacterial isolates, was measured in mm by a ruler. Three replications were maintained for each strain. All *in vitro* antagonism assays were conducted in three independent trials, each with three replications per treatment. Similarly, pot experiments

were arranged in a completely randomised design with three replications per treatment, and data were analysed based on mean values across replications. Per cent growth inhibition [%] of the pathogen was determined by the following method of Jiang *et al.* (2015) with modifications.

The formula for the growth inhibition assay is as follows:

$$\text{Growth inhibition [\%]} = \frac{\text{Diameter of halozone} - \text{Diameter of bacterial colony}}{\text{Diameter of halozone}} \times 100$$

*Net house experiments:* The net house experiment followed a completely randomised design (CRD) with three replications, each consisting of 10 plants per treatment. Fresh and healthy 14-day-old tomato seedlings were transplanted into the pots. For application of treatments, each plant was drenched with 500 mL of a mixture consisting of liquid broth of different bacteria ($10^9$ CFU/mL) with compost tea/vermicompost tea @ 3:1 ratio just after transplanting. Subsequent treatment application was repeated at every 7-day interval till harvesting (Mengesha *et al.* 2017). While drenching with sterile water was done for control plants. Similarly, pathogenic bacteria, *Ralstonia solanacearum,* were applied @ 200ml ($10^9$ CFU/mL) by drenching at 7 days after transplanting. Finally, disease incidence and disease severity index were estimated at 30, 60 and 90 days after transplanting, following the formula of Ayana *et al.* (2011). Data were analysed using ANOVA, and treatment means were separated using the *LSD* test at 5% probability level ($p < 0.05$).

*Biochemical Analysis*

*Proline Estimation*

A protocol by Carillo and Gibon (2011) was followed for the estimation of proline content in plants with some modifications. Five grams of fresh leaf samples were homogenised using 3 mL of 80% ethanol. After the filtration of the resultant homogenate, it was then combined with 1 mL of reaction mixer (Reaction mix: ninhydrin 1% (w/v) in acetic acid 60% (v/v), ethanol 20% (v/v) in a 2 mL Eppendorf tube. After being sealed, the tubes were heated to 95°C for 90 minutes in a water bath. Following cooling at room temperature, the contents of the tube were vortexed and transferred into a 1.5-mL cuvette. The absorbance of the coloured solution was measured at 520 nm.

The following equation was used to calculate proline content:

$$\text{Proline}\left(\frac{\mu\text{mol}}{\text{g}}\right) = \frac{(\text{Absextract-Absblank})}{\text{Slope}} \times \frac{\text{Volume of extract}}{\text{Volume of aliqout}} \times \frac{1}{\text{FW}}$$

*Photosynthetic Pigments Estimation*

For the estimation of *chlorophyll* contents, a test tube containing 5 mL of 95% ethanol was filled with about 0.5 g of fresh leaves. The test tubes were placed in a dark room covered with aluminium foil for seven days. The sediments were then vortexed using a vortex mixer and allowed to settle at the bottom of the test tubes. At 664.2 and 648.6 nm wavelengths, the absorbance was determined using 95% ethanol as a blank. Lichtenthaler's (1987) methods were used to express the total *chlorophyll* content as mg/g FW.

Calculations:

$$\textit{Chl a}\ [\text{mg/g}] = \frac{(13.36 \times \text{A664.2} - 5.19 \times \text{A648.6}) \times \text{Volume of solvent [mL])}}{\omega\ [\text{g}] \times 1000}$$

$$\textit{Chl a}\ [\text{mg/g}] = \frac{(27.43 \times \text{A648.6} - 8.12 \times \text{A664.2}) \times \text{Volume of solvent [mL])}}{\omega\ [\text{g}] \times 1000}$$

$$\textit{Total Chl a}\ [\text{mg/g}] = \frac{(5.24 \times \text{A664.2} - 22.24 \times \text{A648.6}) \times \text{Volume of solvent [mL])}}{\omega\ [\text{g}] \times 1000}$$

Data were recorded, and the diameter of the inhibition zone was noted.

*Total Antioxidant Capacity Estimation*

Two millilitres of the extract were mixed with one millilitre of the reagent solution, which contained 0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. After being sealed, the tubes were placed in a water bath at 50°C for 90 minutes. After the samples had cooled to room temperature and were vortexed, the absorbance of each solution was measured at 695 nm using a spectrophotometer against a reagent blank. Total antioxidant capacity was expressed as ascorbic acid equivalents (AAE), measured in mg/g of dry material (Prieto *et al. 1*999).

*Malondialdehyde* (*MDA) Estimation*

To homogenise 0.1 g of leaf tissue, 2 mL of 0.1% TCA was utilised. For 20 minutes, the homogenate was centrifuged at 10,000 × g. Five hundred microlitres of the supernatant were added with 2 millilitres of 20% TCA mixed with 0.5% TBA. Following 15 minutes of heating at 95°C, the mixture was cooled on ice, and the absorbance was measured at 532 nm

(Heath & Packer 1968). The nonspecific absorption value at 600 nm was subtracted.

*Statistical Data Analysis*

Data for both *in vitro* and pot experiments were statistically analysed using Statistix 10 software. Analysis of variance (ANOVA) was performed, and treatment means were separated by the least significant difference (*LSD*) test at a 5% level of significance ($p < 0.05$).

## RESULTS

*Antagonism Assay*

Antagonistic bacteria were screened out against *R. solanacearum* in three different media. A total of 21 bacterial isolates were evaluated, of which 14 isolates showed antagonistic activity against the pathogen in all these cases, which were confirmed as *Bacillus* by molecular identification. Data were recorded at 2, 4 and 6 days after incubation (DAI), and the diameter of the inhibition zone was noted in cm.

In case of lysogeny agar media, among all the antagonistic isolates, $B_1$, $B_2$ and $B_3$ showed the highest growth inhibition, 86.95%, 83.87% and 78.54% respectively, compared to the control treatment at 6 DAI (Figure 1a, 2). Again, in vermicompost tea media, the highest growth inhibition, 87.68%, 84.18% and 84.18% having the inhibition zones of 4.06 cm, 3.16 cm and 3.16 cm, were recorded at 6 DAI in $B_1$, $B_2$ and $B_3$ compared to the respective control (Figure 1b, 2). Similar results were also found when an antagonism assay was performed at compost tea media at 2, 4 and 6 DAI (Figure 1c, 2).

*Effect of Different Treatments on Bacterial Wilt Disease After Soil Application*

The data on disease incidence and disease severity varied significantly among the treatments on 30, 60 and 90 DAT. Control treatment ($T_1$ = soil only) had a higher mean than $T_{12}$ (Bactroban, chemical control), $T_{10}$ (soil + vermicompost tea + $B_1$ + $B_2$ + $B_3$) and $T_4$ (soil + vermicompost tea + $B_2$) treatments, which showed a lower disease suppression ability than the combination treatments and chemical.

At each time period, all the treatments performed better than the control, $T_1$ (control), at every data collection period. The lowest value for both disease incidence and severity was found in $T_{12}$ (Bactroban). However, among biological treatments, statistically similar disease suppressive effect with $T_{12}$ (Bactroban) was achieved by both $T_{10}$ (soil + vermicompost tea + $B_1$ + $B_2$ + $B_3$) and $T_4$ (soil + vermicompost tea + $B_2$) throughout the experimental session. For $T_{10}$

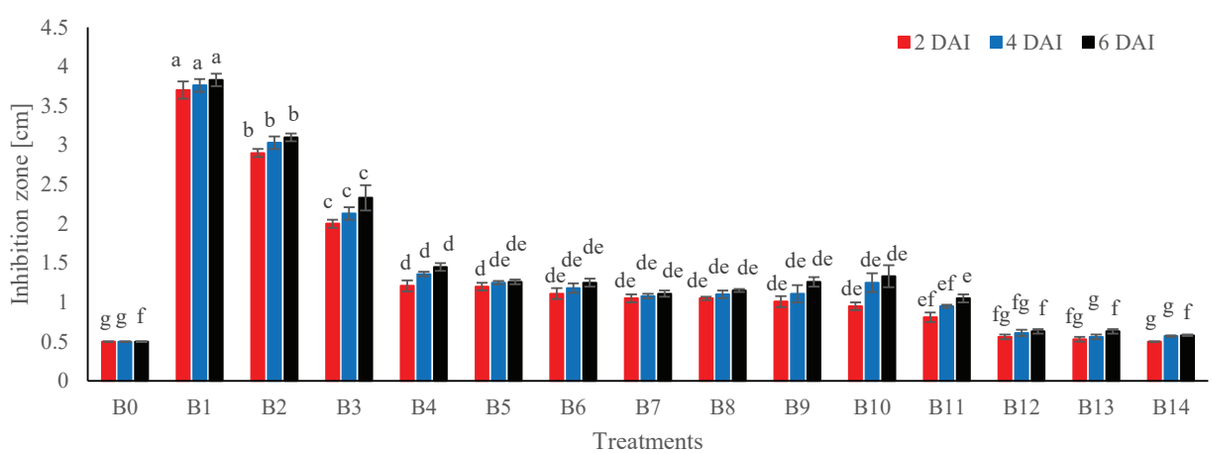


Figure 1a. *In vitro* antagonistic activity of different *Bacillus* isolates against *Ralstonia solanacearum* on lysogeny agar medium at 2, 4 and 6 days after inoculation (DAI). Bars represent the mean inhibition zone diameter [cm] ± standard error. Different lowercase letters (a – g) above the bars indicate statistically significant differences among treatments at each observation time according to the least significant difference (*LSD*) test at $p < 0.05$, while bars sharing the same letter are not significantly different.

Note: $B_0$ – control (without *Bacillus*); $B_1$ – *Bacillus megaterium* (B40DhanSat); $B_2$ – *Bacillus cereus* (BD49IslamSat); $B_3$ – *Bacillus cereus* (EB36KalSat); $B_4$ – *Bacillus cereus* (BDISOB08KhuDu); $B_5$ – *Bacillus amyloliquefaciens* (SH 44E2); $B_6$ – *Bacillus aquimaris* (B21BurirBgr); $B_7$ – *Bacillus licheniformis* (SH10E1); $B_8$ – *Bacillus subtilis* (BDISOB109KhuBa); $B_9$ – *Bacillus subtilis* (SH 44E4); $B_{10}$ – *Bacillus flexus* (B22ChilaBgr); $B_{11}$ – *Bacillus flexus* (B43TalaSat); $B_{12}$ – *Bacillus cereus* (EB28NachSat); $B_{13}$ – *Bacillus subtilis* (LYSX73); $B_{14}$ – *Bacillus cereus* (SH41E2).

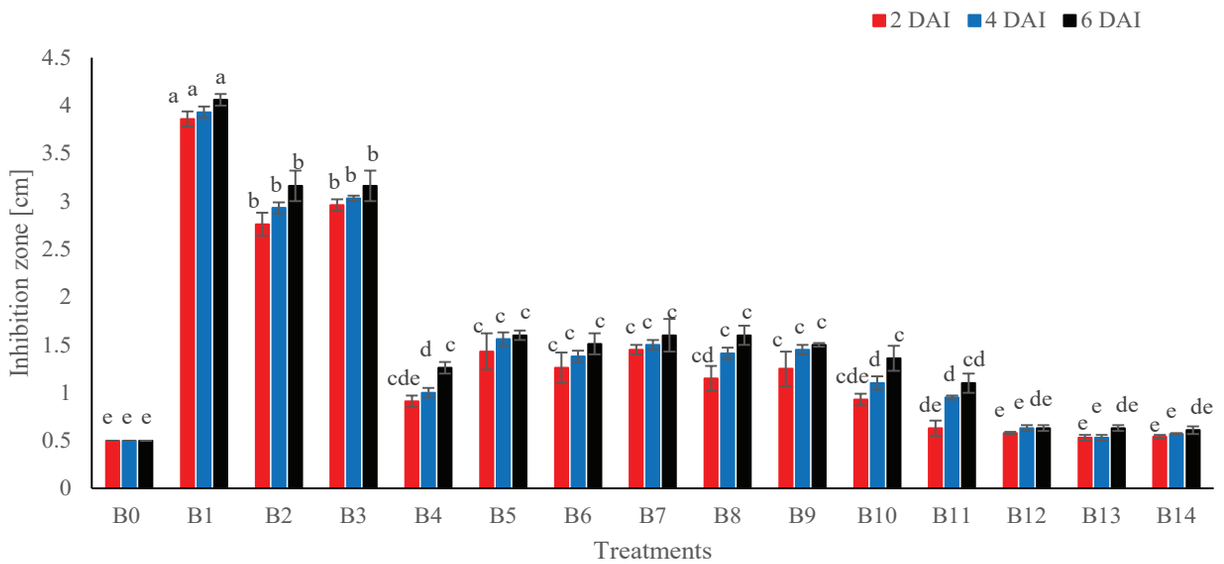


Figure 1b. *In vitro* antagonistic activity of vermicompost tea medium supplemented with different *Bacillus* isolates against *Ralstonia solanacearum* at 2, 4 and 6 days after inoculation (DAI). Bars represent the mean inhibition zone diameter [cm] ± standard error. Different lowercase letters (a – f) above the bars indicate statistically significant differences among treatments at each observation time according to the least significant difference (*LSD*) test at $p < 0.05$; bars sharing the same letter are not significantly different.

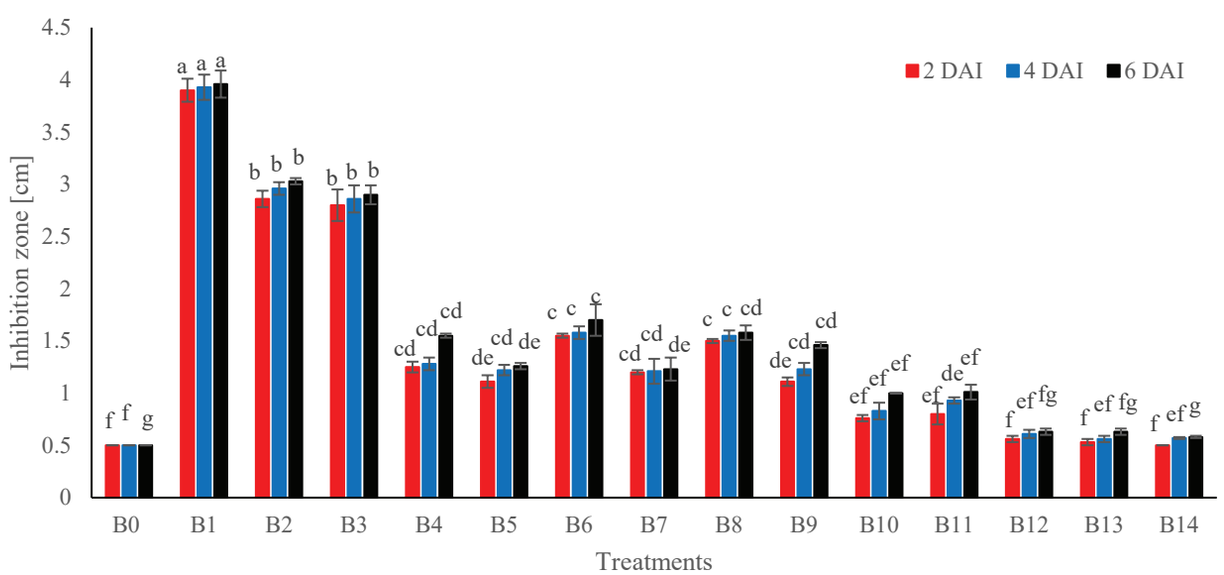


Figure 1c. *In vitro* antagonistic activity of compost tea medium supplemented with different *Bacillus* isolates against *Ralstonia solanacearum* at 2, 4 and 6 days after inoculation (DAI). Bars represent the mean inhibition zone diameter [cm] ± standard error. Different lowercase letters (a – f) above the bars indicate statistically significant differences among treatments at each observation time according to the least significant sifference (*LSD*) test at $p < 0.05$; bars sharing the same letter are not significantly different.

(soil + vermicompost tea + $B_1$ + $B_2$ + $B_3$), disease incidence was found as 8.00%, 8.63% and 8.93% with 7.90%, 8.33% and 8.67% disease severity, respectively, at 30, 60 and 90 DAS. While $T_4$ (soil + vermicompost tea + $B_2$) showed 8.17%, 10.33% and 11.17% disease incidence and 8.00%, 9.16% and 10.16% disease severity at data collection periods (Figure 3a, 3b).

A gradual decline in disease incidence and severity at 90 DAT compared to earlier assessments (control treatment) was observed. This reduction is attributed to the cumulative effects of repeated applications of bio-enriched compost/vermicompost teas, the progressive colonisation of Bacillus isolates in the rhizosphere, and the enhanced physiological defences of plants at later growth stages.

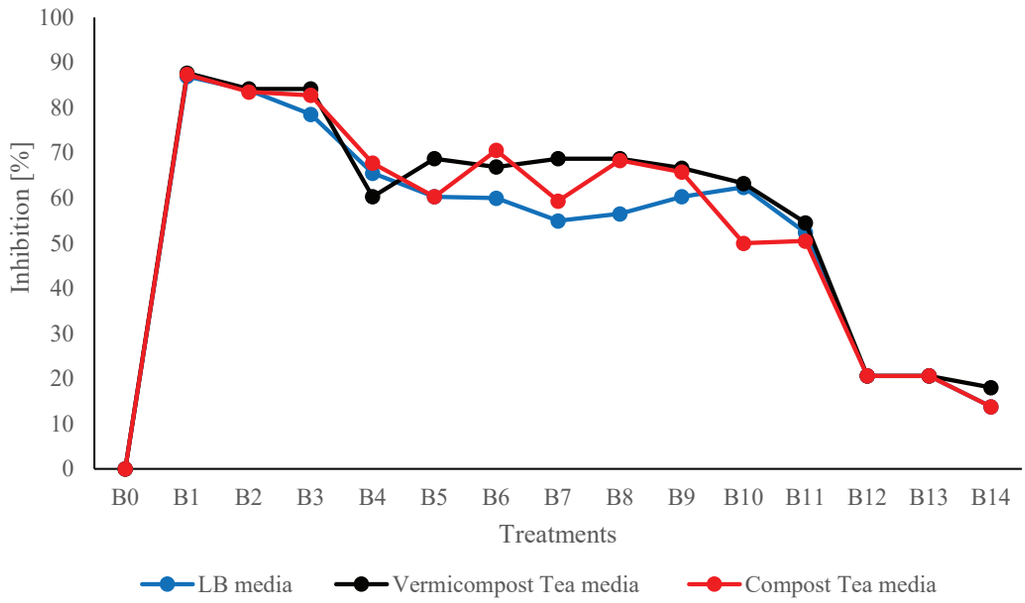


Figure 2. Inhibition percentage of bacterial isolates in three different media.

*Effect of Treatments on Growth Parameters of Tomato*

Data on several growth-promoting factors such as plant height, no. of leaflets, shoot length, fresh weight, dry weight of shoot, root length, and fresh weight, dry weight of root were recorded at different DAT (Table 2, 3, 4, and 5). Compared to the untreated control in treatment $T_1$, where no supplements were administered, all of the other treatments considerably enhanced the growth promotion of the tomato plant.

A significant increment in the plant height by 82.20%, 120% was found at 30 and 60 DAT, respectively, compared to the untreated control. At 90 DAT, plant height was increased about 1.79 times compared to the control treatment (Table 2). Again at 30, 60 and 90 DAT, the highest no. of leaflets recorded in treatment $T_{10}$ (soil+vermicompost tea+B1+B2+B3) was 15.67, 24.33 and 26, respectively, which showed an enhancement of leaflets from 116.00–147.55% compared to the untreated control, $T_1$. Treatment $T_4$ (soil+vermicompost tea $+B_2$) and $T_{12}$ (Bactroban) also showed statistically similar results, whereas other treatments moderately increased the number of leaflets (Table 3).

All other vegetative parameters except plant height and no of leaflets were recorded at 90 DAT, where $T_4$, $T_{10}$ and $T_{12}$ indicated a significant improvement in growth traits compared to the control treatment. In all these cases, $T_4$ had the highest growth promotion where soil was mixed with vermicompost tea and bioagent $B_2$ and identical in increasing the growth promotion with the treatment $T_{10}$ (soil+ vermicompost tea+$B_1$+$B_2$+$B_3$), $T_{12}$ (Bactroban) (Table 4, 5).

*Yield Traits*

Data on yield parameters such as total no. of fruits/plant and total fresh weight of fruit/plant were recorded at 90 DAT. Yield parameters significantly increased under the effect of various treatments. An increment in the total no. of fruit per plant was observed from 36.23% to 199.72% at 90 DAT. Total fresh weight was also found to be significantly increased up to 114.15% from 20.34% which is about 1.20–2.14-fold compared to the control treatment (Figure 4).

*Biochemical Traits*

Data on biochemical parameters were analysed to assess the effect of different treatments on proline, MDA, total antioxidant capacity (TAC) and *chlorophyll*. Proline content was found to be higher in the plant treated with $T_4$ treatment, where a combined application was done in soil with vermicompost tea and biocontrol agent ($B_2$). Proline content was increased by 213.79% which is about 3.13-fold higher compared to the control treatment. Treatment $T_{10}$ and $T_{12}$ were also consistent and statistically similar to $T_4$ (Figure 5a).

Table 2

Influence of various treatments on rice plant growth at different growth stages (30, 60 and 90 DAT)

| Treatments | Plant height [cm] | | |
|---|---|---|---|
| | 30 DAT | 60 DAT | 90 DAT |
| $T_1$ (control) | 24.33 ± 2.03$^{d}$ | 36.00 ± 1.00$^{d}$ | 46.33 ± 1.86$^{d}$ |
| $T_2$ | 35.67 ± 0.33$^{c}$ | 55.33 ± 0.33$^{b}$ | 65.00 ± 0.00$^{c}$ |
| $T_3$ | 35.67 ± 0.67$^{c}$ | 60.17 ± 2.59$^{b}$ | 75.67 ± 0.67$^{b}$ |
| $T_4$ | 44.33 ± 0.88$^{a}$ | 79.33 ± 0.33$^{a}$ | 83.00 ± 1.53$^{a}$ |
| $T_5$ | 37.00 ± 1.00$^{bc}$ | 60.83 ± 0.60$^{b}$ | 66.00 ± 2.08$^{c}$ |
| $T_6$ | 27.00 ± 1.53$^{d}$ | 55.67 ± 0.67$^{b}$ | 63.33 ± 0.33$^{c}$ |
| $T_7$ | 35.67 ± 0.33$^{c}$ | 60.17 ± 0.17$^{b}$ | 74.67 ± 2.60$^{b}$ |
| $T_8$ | 30.00 ± 1.53$^{cd}$ | 60.67 ± 0.67$^{b}$ | 74.00 ± 0.58$^{b}$ |
| $T_9$ | 24.67 ± 1.76$^{d}$ | 46.33 ± 0.88$^{c}$ | 53.33 ± 1.67$^{d}$ |
| $T_{10}$ | 44.33 ± 2.60$^{a}$ | 79.00 ± 0.33$^{a}$ | 83.00 ± 0.58$^{a}$ |
| $T_{11}$ | 30.67 ± 0.88$^{cd}$ | 60.67 ± 0.67$^{b}$ | 75.00 ± 0.00$^{b}$ |
| $T_{12}$ | 43.67 ± 0.88$^{ab}$ | 63.33 ± 3.33$^{a}$ | 83.33 ± 1.67$^{a}$ |

a, b, c, d indicates significant differences among treatments based on statistical analysis. Specifically, mean separation was performed using the least significant difference (*LSD*) test at $p < 0.05$ (Statistix 10). Values sharing the same letter are not significantly different, whereas values with. Values sharing the same letter are not significantly different, whereas values with different letters differ significantly at the 5% probability level. Note: DAT – see figure 3.

Table 3

Effect of different treatments on vegetative growth of tomato at different days after transplantation (DAT)

| Treatments | Number of leaflets | | |
|---|---|---|---|
| | 30 DAT | 60 DAT | 90 DAT |
| $T_1$ (control) | 6.33 ± 0.33$^{d}$ | 11.00 ± 0.58$^{g}$ | 12.00 ± 0.57$^{d}$ |
| $T_2$ | 11.00 ± 0.57$^{b}$ | 17.00 ± 0.58$^{bcd}$ | 17.66 ± 0.33$^{b}$ |
| $T_3$ | 11.67 ± 0.67$^{b}$ | 15.33 ± 0.88$^{cde}$ | 16.66 ± 0.88$^{bc}$ |
| $T_4$ | 15.33 ± 0.33$^{a}$ | 24.00 ± 1.00$^{a}$ | 25.00 ± 0.57$^{a}$ |
| $T_5$ | 11.33 ± 0.33$^{b}$ | 18.33 ± 0.33$^{bc}$ | 19.00 ± 0.57$^{b}$ |
| $T_6$ | 10.00 ± 0.58$^{bc}$ | 12.00 ± 0.58$^{g}$ | 13.33 ± 0.33$^{d}$ |
| $T_7$ | 9.67 ± 0.88$^{bcd}$ | 14.33 ± 0.67$^{def}$ | 14.66 ± 0.33$^{cd}$ |
| $T_8$ | 10.67 ± 1.20$^{b}$ | 13.67 ± 0.67$^{efg}$ | 14.33 ± 0.33$^{cd}$ |
| $T_9$ | 6.67 ± 0.88$^{cd}$ | 11.33 ± 0.67$^{fg}$ | 13.00 ± 0.57$^{d}$ |
| $T_{10}$ | 15.67 ± 0.33$^{a}$ | 24.33 ± 0.67$^{a}$ | 26.00±0.57$^{a}$ |
| $T_{11}$ | 10.00 ± 0.58$^{bc}$ | 18.67 ± 0.33$^{b}$ | 19.00 ± 0.57$^{b}$ |
| $T_{12}$ | 15.67 ± 0.67$^{a}$ | 23.33 ± 0.33$^{a}$ | 23.66 ± 0.33$^{a}$ |

a, b, c, d, e, f, g indicates significant differences among treatments based on statistical analysis. Specifically, mean separation was performed using the least significant difference (*LSD*) test at $p < 0.05$ (Statistix 10). Values sharing the same letter are not significantly different, whereas values with. Values sharing the same letter are not significantly different, whereas values with different letters differ significantly at the 5% probability level.

The level of MDA remarkably increased in the control treatment, $T_1$. However, the combined application of vermicompost and bioagent with soil in treatment $T_{10}$ and $T_4$ reduced the MDA level in plant leaves by 63.36% and 55.44% compared to the untreated control. On the other hand, TAC was found to be higher by 94.485% and 57.48% in $T_4$ and $T_{10,}$ respectively, in comparison to control, $T_1$ (Figure 5a).

As photosynthetic efficacy is related to the plant biomass, the levels of *chlorophyll a, b* and *c* were determined in tomato plants in this experiment. An increment in *chlorophyll a, b* and *c* content was found in $T_4$ by 137.5%, 116.67% and 126.92% which is 0.11, 2.16 and 2.26 times more than the control. $T_{10}$ and $T_{12}$ were also statistically similar to this result, but $T_4$ showed the highest *chlorophyll* content in tomato leaves (Figure 5b).

## DISCUSSION

Tomato is an important crop from an economic point of view, but it is affected by various bacterial diseases, which pose a major constraint in the production sector (Ji *et al.* 2005). The bacterial wilt pathogen can persist in plant debris for a considerable period of time, posing a challenge for farmers (McCarter 1979; Granada & Sequeira 1983). As effective approaches for managing this disease are limited, this experiment was initiated to evaluate the efficiency of compost and vermicompost extract, together with biological agents, as an alternative method.

A dual culture antagonism assay (disc diffusion method) was performed, having 21 *Bacillus* isolates, where 14 isolates showed varying degrees of inhibition of *R. solanacearum*. According to Shekhawat *et al*. (1993), *Bacillus* species were found to exhibit the highest inhibitory zone *in vitro* against

Table 4

Effect of different treatments on vegetative growth of tomato (shoot length, fresh and dry weight of shoot)

| Treatments | Shoot length [cm] | Fresh weight [gm] | Dry weight [gm] |
|---|---|---|---|
| $T_1$ (control) | 46.33 ± 1.86$^{d}$ | 40.67 ± 0.67$^{g}$ | 12.44 ± 0.68$^{g}$ |
| $T_2$ | 65.00 ± 0.00$^{c}$ | 92.67 ± 1.45$^{b}$ | 19.73 ± 0.69$^{cde}$ |
| $T_3$ | 75.67 ± 0.67$^{b}$ | 77.33 ± 0.88$^{d}$ | 20.69 ± 0.85$^{cd}$ |
| $T_4$ | 83.33 ± 1.67$^{a}$ | 112.33 ± 1.45$^{a}$ | 39.27 ± 0.43$^{a}$ |
| $T_5$ | 66.00 ± 2.08$^{c}$ | 83.00 ± 1.15$^{c}$ | 17.58 ± 0.71$^{def}$ |
| $T_6$ | 63.33 ± 0.33$^{c}$ | 67.00 ± 0.58$^{e}$ | 15.82 ± 0.16$^{f}$ |
| $T_7$ | 74.67 ± 2.60$^{b}$ | 94.33 ± 0.33$^{b}$ | 20.92 ± 0.04$^{bcd}$ |
| $T_8$ | 74.00 ± 0.58$^{b}$ | 59.67 ± 0.33$^{f}$ | 24.10 ± 0.21$^{b}$ |
| $T_9$ | 53.33 ± 1.67$^{d}$ | 84.33 ± 0.67$^{c}$ | 21.78 ± 0.91$^{bc}$ |
| $T_{10}$ | 83.00 ± 0.58$^{a}$ | 110.33 ± 1.45$^{a}$ | 38.23 ± 0.62$^{a}$ |
| $T_{11}$ | 75.00 ± 0.00$^{b}$ | 75.00 ± 1.15$^{d}$ | 16.92 ± 0.99$^{ef}$ |
| $T_{12}$ | 83.00 ± 1.53$^{a}$ | 112.00 ± 1.53$^{a}$ | 38.83 ± 0.75$^{a}$ |

a, b, c, d, e, f, g indicates significant differences among treatments based on statistical analysis. Specifically, mean separation was performed using the least significant difference (*LSD*) test at $p < 0.05$ (Statistix 10). Values sharing the same letter are not significantly different, whereas values with. Values sharing the same letter are not significantly different, whereas values with different letters differ significantly at the 5% probability level.

Table 5

Effect of different treatments on vegetative growth of tomato (root length, fresh and dry weight of root)

| Treatments | Root length [cm] | Fresh weight [gm] | Dry weight [gm] |
|---|---|---|---|
| $T_1$ (control) | 23.50 ± 0.76$^{h}$ | 9.17 ± 0.44$^{e}$ | 4.53 ± 0.27$^{g}$ |
| $T_2$ | 33.67 ± 0.33$^{ef}$ | 15.83 ± 0.60$^{ab}$ | 8.10 ± 0.15$^{bcd}$ |
| $T_3$ | 30.83 ± 0.44$^{fg}$ | 14.00 ± 0.58$^{bc}$ | 6.90 ± 0.10$^{def}$ |
| $T_4$ | 44.33 ± 0.33$^{a}$ | 17.17 ± 0.44$^{a}$ | 9.83 ± 0.17$^{a}$ |
| $T_5$ | 41.17 ± 0.44$^{b}$ | 10.50 ± 0.29$^{de}$ | 6.50 ± 0.11$^{def}$ |
| $T_6$ | 29.00 ± 0.58$^{g}$ | 11.33 ± 0.88$^{de}$ | 5.98 ± 0.30$^{fg}$ |
| $T_7$ | 34.83 ± 0.93$^{de}$ | 10.17 ± 0.60$^{de}$ | 6.43 ± 0.29$^{ef}$ |
| $T_8$ | 27.83 ± 1.01$^{g}$ | 9.83 ± 0.17$^{de}$ | 6.90 ± 0.59$^{def}$ |
| $T_9$ | 28.83 ± 0.60$^{g}$ | 11.50 ± 0.29$^{cde}$ | 6.40 ± 0.23$^{ef}$ |
| $T_{10}$ | 40.67 ± 0.44$^{b}$ | 16.97 ± 0.55$^{a}$ | 9.33 ± 0.17$^{ab}$ |
| $T_{11}$ | 37.50 ± 0.29$^{cd}$ | 11.83 ± 0.60$^{cd}$ | 7.83 ± 0.20$^{cde}$ |
| $T_{12}$ | 39.17 ± 0.44$^{bc}$ | 15.50 ± 0.29$^{ab}$ | 8.10 ± 0.44$^{abc}$ |

a, b, c, d, e, f, g indicates significant differences among treatments based on statistical analysis. Specifically, mean separation was performed using the least significant difference (*LSD*) test at $p < 0.05$ (Statistix 10). Values sharing the same letter are not significantly different, whereas values with. Values sharing the same letter are not significantly different, whereas values with different letters differ significantly at the 5% probability level.

*R. solanacearum.* An *in vitro* antagonism assay was performed in lysogeny agar media, compost tea, as well as vermicompost tea media. Three rhizosphere *Bacillus* isolates, $B_1$ (*Bacillus megaterium*), $B_2$ (*Bacillus cereus*) and $B_3$ (*Bacillus cereus*) showed the highest inhibition zone against the pathogen. Mycelial growth was found to be reduced by the presence of compost and vermicompost tea (Tian & Zheng 2013; Mistry & Mukherjee 2015). Moreover, the inhibition zone in vermicompost tea media was found to be more than in compost tea media and lysogeny agar media in this experiment. These findings had similarities with the results demonstrated by Helmy and Abu-Hussien (2024), who proved that vermicompost extract had a strong antagonistic potential against pathogens. It is known that the degree of inhibition zone development during antagonism assay is related to the organism's ability to inhibit *R. solanacearum,* and the inhibition by the *Bacillus* isolates demonstrates their innate capacity to generate some compounds (Sivakumar *et al*. 2014). Antagonistic action of *Bacillus* isolates against specific pathogens *in vitro* is also due to the synthesis of siderophores that play a crucial part in biocontrol, pathogen inhibition, root shoot growth and plant nutrition (Rahman *et al*. 2007; 2021; Amalraj *et al.* 2012).

In the pot experiment, 12 treatments were evaluated at 30, 60 and 90 DAT based on several vegetative and yield parameters. The lowest % disease incidence and severity (7.53% and 7.37%) were observed in $T_{12}$ (chemical). Statistically similar results were found in $T_4$ (8.17% and 8.00%) and $T_{10}$ (8.00% and 7.9%) at 90 DAT, where *Bacillus megaterium* and *Bacillus cereus* were used as a combined application of treatments. According to some research reports, *Bacillus* strains increase the content of humic compounds and total organic carbon in compost by slowing down the metabolism of amino acids and carbohydrates, which helps in disease suppression by compost maturation (Tannouri *et al*. 2022). Strains of *Bacillus* produce lipopeptides (LP), which are antibiotics and help bacteria in moving towards

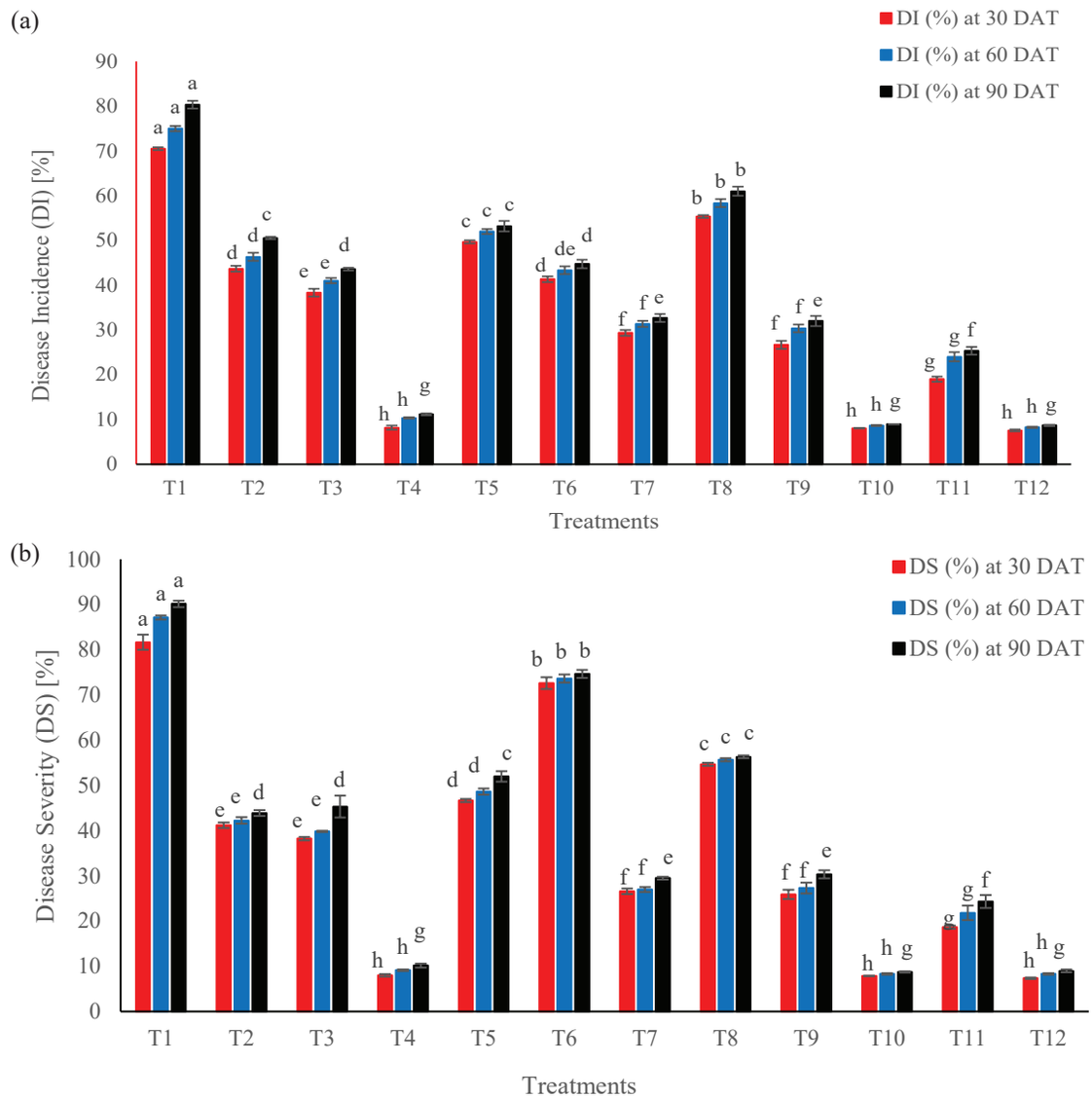


Figure 3. Graphs shown in (a) and (b) indicate the disease incidence [%] and severity index [%] of tomato bacterial wilt respectively at 30, 60 and 90 days after transplantation (DAT). Bars represent disease severity index [%] ± standard error. Different lowercase letters (a – f) above the bars indicate statistically significant differences among treatments at each observation time according to the least significant difference (*LSD*) test at $p < 0.05$; bars sharing the same letter are not significantly different.

Note: $T_1$ – soil (control); $T_2$ – soil + vermicompost tea; $T_3$ – soil + vermicompost tea + $B_1$; $T_4$ – soil + vermicompost tea + $B_2$; $T_5$ – soil + vermicompost tea + $B_3$; $T_6$ – soil + compost tea; $T_7$ – soil + compost tea + $B_1$; $T_8$ – soil + compost tea + $B_2$; $T_9$ – soil + compost tea + $B_3$; $T_{10}$ – soil + vermicompost tea + $B_1$ + $B_2$ + $B_3$; $T_{11}$ – soil + compost tea + $B_1$ + $B_2$ + $B_3$; $T_{12}$ – Bactroban (chemical).

plant secretions for root colonisation (Cao *et al.* 2018). The net house experiment was conducted in sandy loam soil, which is common in vegetable production areas of Bangladesh. This soil type is also representative of conditions in other regions where *Bacillus* spp. have been reported as effective biocontrol agents, such as loamy and sandy loam soils in tropical and subtropical zones. Such soils not only favour pathogen development but also provide conducive conditions for *Bacillus* colonisation and activity, thereby supporting the broader applicability of the current results.

In this study, the *Bacillus* antagonists that performed better in the pot experiment were previously isolated from the rhizosphere zone. Rhizosphere bacteria are an excellent source of biocontrol agents as they can act against pathogens, having a first line of defence system (Sivakumar *et al.* 2014). Another report showed the involvement of induced systemic resistance (ISR) by *Bacillus* for the growth promotion in plants by secreting volatile compounds,

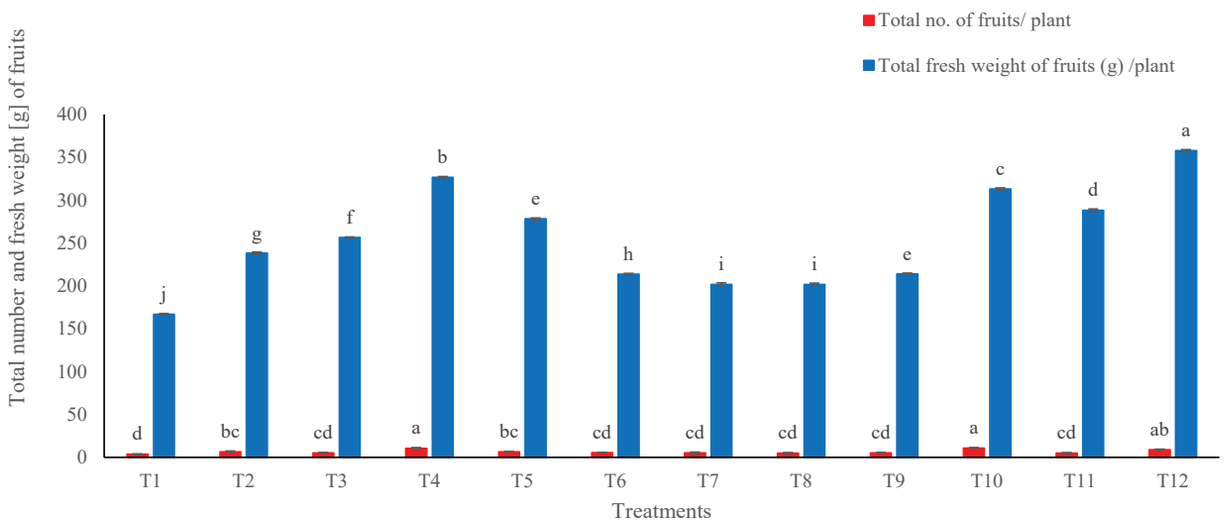


Figure 4. Response of tomato yield components to different treatments. a, b, c, d, e, g, h, i indicates significant differences among treatments based on statistical analysis. Specifically, mean separation was performed using the least significant difference (*LSD*) test at $p < 0.05$ (Statistix 10). Values sharing the same letter are not significantly different, whereas values with. Values sharing the same letter are not significantly different, whereas values with different letters differ significantly at the 5% probability level.

which in turn is responsible for disease incidence reduction, increased plant biomass for sustainable management of disease (Aliye *et al.* 2008).

*B. megaterium* has been reported to promote growth, like root length and shoot length of tomato plants, by producing $GA_3$ (Gibberellic acid) and IAA (Indole-3-acetic acid) in moderation. $GA_3$ helps in plant growth, while IAA aids in organogenesis and cellular regulation through an essential signalling function (Amalraj *et al*. 2012). Application of *B. megaterium* through soil was reported to highest wilt reduction in tomato plants. *B. megaterium* can multiply and persist more in the tomato rhizosphere by biofilm and antibacterial antibiotics production, which results in maximum disease suppression (Sivakumar *et al.* 2014). *B. megaterium* solubilises phosphate, which is associated with nitrogen uptake, which indirectly helps in growth enhancement (Amalraj *et al*. 2012).

Because of antagonistic properties and secondary metabolite production, *Bacillus cereus* is employed as a biocontrol agent against R. solanacearum (Seleim *et al.* 2024). *Bacillus cereus* uses the synthesis of hydrogen cyanide, IAA, salicylic acid, and siderophores as a growth promotion mechanism (Seleim *et al*. 2024). Accumulation of callose and reactive oxygen species (ROS) production is promoted by *Bacillus cereus,* which ultimately helps in the defence mechanism (Lin *et al.* 2020).

Soil amendment by vermicompost was reported to reduce disease incidence and severity of wilt disease of tomato because of the presence of effective biocontrol agents. Antibiosis, hyperparasitism and competition are linked to disease suppression mechanisms. (Haruna 2021). Along with disease parameters, some vegetative and reproductive parameters were also recorded in this study. In most cases, $T_{12}$ (chemical control) exhibited the superior performances at different growth stages, whereas statistically similar and the highest results considering all parameters were observed in $T_4$ (soil + vermicompost tea + $B_2$) and in $T_{10}$ (soil + vermicompost tea + $B_1$ + $B_2$ + $B_3$) among the bio-enriched treatments. Results of the current study are in accordance with the report of Helmy & Abu-Hussien (2024), who reported highly significant variations in root length, shoot length, dry weight, pod diameter and pod weight among the treatments where application of vermicompost tea is done after bio enrichment with microorganisms. Application of vermicompost and compost tea under a pot experiment was reported to increase leaf number, weight of dry matter, yield, root length, and root dry weight (Wang *et al*. 2019; Alkobaisy *et al*. 2021). Application of vermicompost tea showed higher growth promotion in plants than compost tea.

Various biopesticidal microbes present in compost teas have been found to function as natural pes-

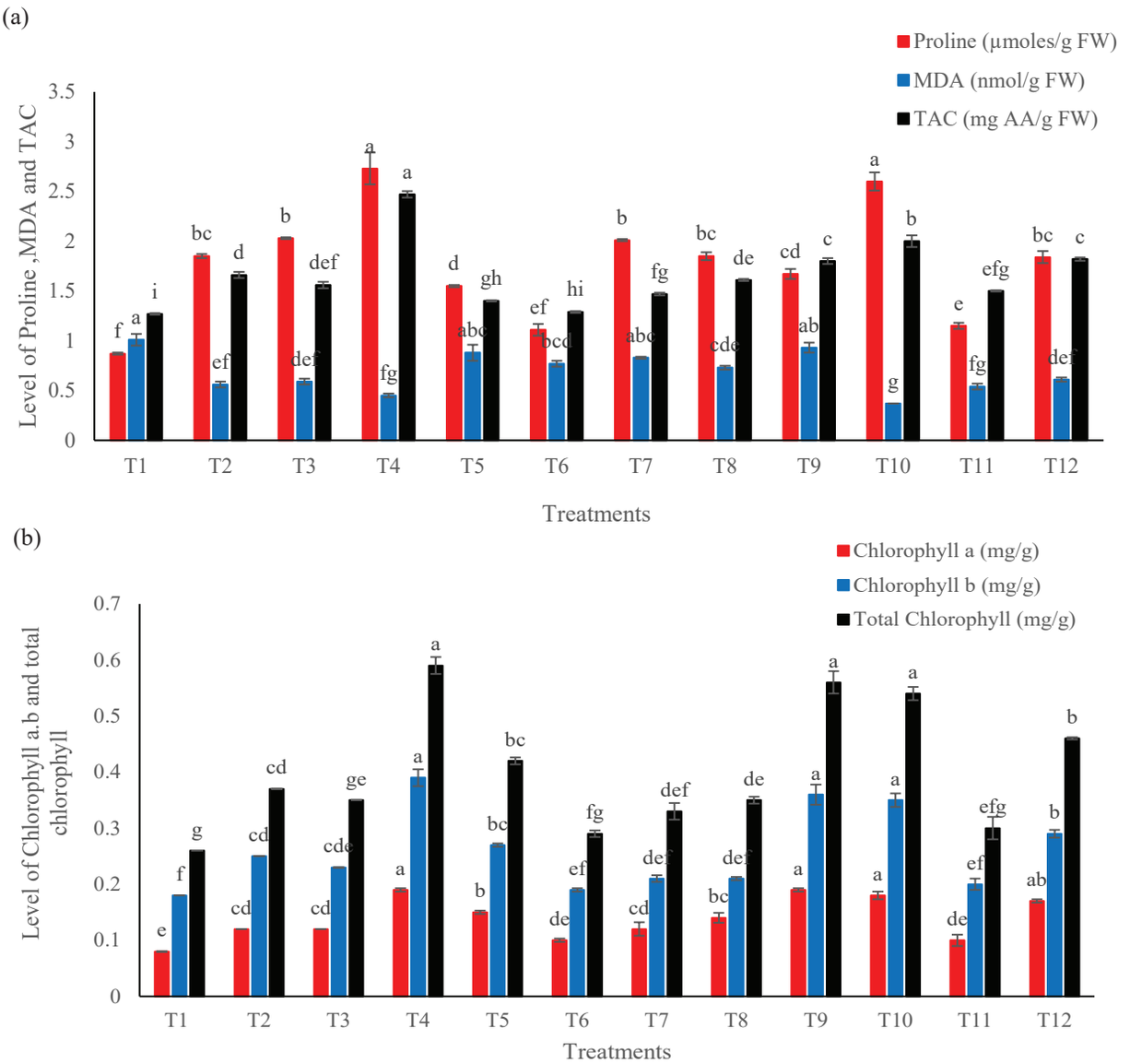


Figure 5. Levels of biochemical parameters (a) proline, MDA and TCA (b) *Chlorophyll a, b* and total *chlorophyll* respectively. a, b, c, d, e, f, g indicates significant differences among treatments based on statistical analysis. Specifically, mean separation was performed using the least significant difference (*LSD*) test at $p<0.05$ (Statistix 10). Values sharing the same letter are not significantly different, whereas values with. Values sharing the same letter are not significantly different, whereas values with different letters differ significantly at the 5% probability level.

ticides (Gomez-Brandon *et al*. 2015). Throughout the composting process, a range of microorganisms successfully convert the organic substrate biochemically (Awasthi *et al.* 2017). Compost has been used to increase the antagonistic activity of several bacteria and induce resistance, which has been reported to manage soil-borne diseases (Haruna 2021). Through a variety of antagonistic activity mechanisms, microbial compounds produced in compost tea help in pathogen suppression by reducing conidial germination (Gomez-Brandon *et al*. 2015). Compost tea may also provide soils with soluble mineral nutrients, organic acids and growth regulators (Gomez-Brandon *et al*. 2015; Wang *et al*. 2019).

A good number of useful bacteria, as well as nutrients, are also present in vermicompost extract. The advantages of vermicompost tea over compost tea are that worms present in vermicompost secrete chemicals that kill the pathogen, making it safe for plants. Again, to maintain the proportion of organic matter, carbon and nitrogen content in soil, tea is considered a better option than filtered liquid. Applica-

tion of vermicompost tea helps in the proper growth and increased yield of plants due to higher enzymatic action, beneficial microbial diversity in soil (Wang *et al.* 2019). Growth and yield are correlated with better nutrient uptake because of the presence of humic acid, which improves the physical characteristics of soil by facilitating microbes and solubilising essential nutrients required for plant growth when vermicompost levels are higher (Chatterjee *et al.* 2006; Sarma *et al*. 2010). The earthworms' intestinal contribution may be the cause of the vermicompost's more active microbial population, which are responsible for noticeably higher levels of dehydrogenase activity in teas (Wang *et al*. 2019). In Bangladesh, the subtropical monsoon climate – with high temperatures and humidity – creates favourable conditions for bacterial wilt development. These climatic conditions are comparable to those in several native regions where *Bacillus* spp. have been isolated and applied as biocontrol agents, such as tropical and subtropical zones of Asia and Africa. This similarity in environmental conditions highlights the potential adaptability and effectiveness of Bacillus-enriched compost and vermicompost teas under local field conditions.

The present study also evaluated some biochemical parameters as plant stress response. $T_4$ (soil + vermicompost tea + $B_2$) treatment showed the highest total *chlorophyll* content (0.59 mg/g) compared to $T_1$ (control), which indicated that vermicompost tea helps in *chlorophyll* accumulation. Doan *et al.* (2013) reported that tomato plants treated with vermicompost exhibited higher *chlorophyll* concentrations compared to plants receiving other organic amendments. *Chlorophyll* content is widely used as an indicator of overall plant health, as reduced levels often reflect nutrient deficiencies or disease (Freidenreich *et al.* 2019). *Chlorophyll a, b,* as well as total *chlorophyll* content, were increased in $T_4$ (soil + vermicompost tea + $B_2$) and $T_{10}$ (soil + vermicompost tea + $B_1$ + $B_2$ + $B_3$) treatments. Nutrients such as nitrogen, phosphorus, and iron are essential for *chlorophyll* production and have previously been detected in vermicompost (Domínguez *et al.* 2013; Lim *et al.* 2014). Additionally, $T_4$ (soil vermicompost tea + $B_2$) exhibited the highest proline accumulation. *Bacillus megaterium* has a protective effect by reducing the need for excessive proline accumulation, which can be detrimental to plant growth (Akram *et al.* 2019). *B. cereus* can also help plants by enhancing antioxidant activities and accumulating proline (Direk *et al.* 2024b).

$T_{10}$ (soil + vermicompost tea + $B_1$ + $B_2$ + $B_3$) showed the lowest MDA levels, indicating reduced oxidative stress under pathogenic conditions. As for TCA, $T_4$ (soil + vermicompost tea + $B_2$) showed the highest antioxidant content (2.47 mg AA/g FW), suggesting that *Bacillus cereus* can reduce MDA levels in tomato plants, which is a key indicator of oxidative stress damage. This reduction is linked to several mechanisms, including increased antioxidant enzyme activity and improved plant defences against stress (Lv *et al.* 2024). Buqori *et al*. (2025) also noted that *B. megaterium* reduces MDA levels in plants by mitigating oxidative stress, promoting antioxidant defence systems. Jabeen *et al.* (2022) reported that inoculation with *B. cereus* enhanced plant biomass, *chlorophyll* content, relative water content, and antioxidant enzyme activity, while simultaneously decreasing MDA accumulation.

## CONCLUSIONS

Based on experiments conducted under both *in vitro* and pot conditions, compost and vermicompost teas fortified with *Bacillus* isolates exhibited strong antagonistic activity against *Ralstonia solanacearum*, thereby demonstrating their potential as effective and eco-friendly biocontrol agents. Specifically, *B. megaterium* and *B. cereus* fortified vermicompost tea showed significantly better performance over compost tea in all cases and helped in the overall growth enhancement and yield of tomato. Therefore, the application of compost and vermicompost extracts fortified with *Bacillus* isolates represents an effective and eco-friendly alternative to chemical control and a long-term method of eco-friendly management of tomato bacterial wilt for the farmers. Future studies could focus on comparing the original rhizosphere of *Bacillus* isolates in their primary habitat with the tomato rhizosphere under greenhouse and field conditions. Such comparisons would help clarify the mechanisms of adaptation and colonisation that underpin their biocontrol efficacy.

***Conflicts of Interest:*** The authors declare that they have no competing interests in this work.

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