

# Effect of different microbial agents on the growth and development of tomato cultivated in substrate

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## Abstract:

Substrate cultivation has become the mainstay of most vegetable cultivation. Many studies have shown that microbial agents can promote the release of nutrients in the cultivation medium and thus meet the needs of crop growth and development. In this study, the effects of six microbial agents on the growth of tomato plants and fruit development under substrate cultivation conditions were investigated using 'Provence' tomato as the material, and the physical and chemical properties of the substrates were measured and analyzed. It was found that the six microbial agents used in this study could promote the nutrients in the substrate, the growth and development of tomato plants, and the improvement of fruit yield and quality to different degrees. In this study, the two microbial agents, *Bacillus subtilis* and *Bacillus cereus*, can promote the release of nutrients in the substrate, the growth of plants, and the conversion of substances in the fruits, and realize the quality of the quality of the yield, and the results of this study can provide a technical guide for the cultivation of tomatoes in an efficient and high-quality substrate.

**Keywords:** Microbial agents; Cultivation substrates; Tomato; *Bacillus subtilis*; *Bacillus cereus*.

## 1. Introduction

Against the backdrop of rapid global population growth, there is an increasing demand for crops worldwide to feed more and more people, so the use of chemical fertilizers and pesticides has become more and more frequent. However, the excessive use of chemical fertilizers and pesticides will lead to a series of problems, such as the destruction of soil structure and the decline of soil activity and microbial abundance<sup>[1]</sup>. In soil ecosystems, soil microorgan-

isms play an essential role in forming soil aggregates, degradation of organic matter, nitrogen fixation, nutrient cycling, photosynthesis, and the production of greenhouse gases. Soil microorganisms are the main drivers of soil ecosystems, and they can enhance soil fertility and ensure sustainable soil development. However, decreasing the number and abundance of soil microorganisms can lead to problems such as soil compaction, salinization, and the proliferation of pathogenic bacteria, reducing crop yields and quality and affecting agriculture's sustainability<sup>[2]</sup>.

### 1.1 Effect of inter-root growth promoting bacteria on plant growth

Rhizobia, mycorrhizae, and fungi are microorganisms that are primarily symbiotic with plants and are also central plant root-promoting bacteria. These microorganisms are commonly found within the plant root system or in the plant root environment. Mineral elements such as nitrogen and phosphorus, as well as various organic nutrients required by plants, can be provided by these microorganisms directly or indirectly, thus promoting plant development and maturation<sup>[3-6]</sup>. At the same time, rhizosphere-promoting bacteria can improve the microbial community structure around the root system, enhance the ability of inter-root nutrient balanced supply, synthesize growth-regulating substances and secrete secondary metabolites, and inhibit the propagation of pathogens, thus improving the yield and quality of crops<sup>[7-9]</sup>. Studies have shown that rhizobacteria can convert airborne nitrogen into ammonia that plants can absorb and utilize, reducing reliance on nitrogen fertilizers, reducing fertilizer application, and improving economic efficiency<sup>[10]</sup>. In addition, most of the inorganic and organic phosphorus in the soil is difficult for the plant root system to absorb.

In contrast, the effective phosphorus content at the inter-root level can be enhanced by being solubilized or transformed by substances such as phosphatases or organic acids secreted by root-promoting bacteria<sup>[11,12]</sup>. Data show that 98% of the potassium is in the soil in an ineffective form. In contrast, potassium-solubilizing bacteria can release soluble potassium by decomposing potassium-containing minerals, thus increasing the amount of potassium available for uptake around the plant's root system<sup>[13]</sup>. In addition, some rhizosphere nutrient bacteria can produce growth regulators such as indole-3-acetic acid, cytokinins, gibberellins, etc., to promote the growth and development of plant roots, thereby improving the absorption and utilization of mineral elements and other nutrients in the soil<sup>[14]</sup>.

### 1.2 Application of microbial fungicides

After microbial agents are added to the soil, they can stimulate the growth of the root system, produce secondary metabolites, and enhance the resilience of plants<sup>[15,16]</sup>. At the same time, microbial fungicides can accelerate the release of nutrients in the cultivation substrate and improve the absorption and utilization of nutrients by the plant root system, thus increasing yield and income<sup>[17]</sup>. Therefore, in the future, microbial fungicides will play an essential role in agriculture's green and sustainable development and will also be an indispensable component in realizing agricultural modernization in China.

### 1.3 Scientific questions and research objectives

In recent years, microbial fungicides have become a hot agricultural topic because microbial fungicides can not only improve soil nutrients but also reduce environmental pollution. At present, microbial agents have been widely used in China. However, most of the studies on the use of microbial agents to promote crop growth and improve yield and quality have been based on soil cultivation conditions. The studies on the use of microbial agents in combination with substrate cultivation methods have been relatively scarce.

In this study, the tomato was used as the test material to study the influence of adding different microbial agents on the physicochemical properties of the cultivation substrate (morel mushroom dregs, grass charcoal, vermiculite mix) as well as the traits of tomato plants and fruit traits, and to screen out the microbial agents that are suitable for substrate cultivation, to contribute to the research on efficient and high-quality substrate cultivation of tomato.

## 2. Experimental materials and methods

### 2.1 Types and sources of experimental tomatoes

Seedlings of tomato 'Provence' variety, composite substrate (morel mushroom dregs: grass charcoal: vermiculite = 1:1:1, v/v), and six microbial agents (*Bacillus subtilis*, *Bacillus cereus*, *Penicillium violet*, BT, *Bacillus suis*, *Bacillus subtilis* (botany)) used in the experiment were purchased from the market.

### 2.2 Experimental Treatments

The experiment was set up with seven treatments, five pots for each treatment, and was replicated three times. Well-grown and uniform tomato seedlings were selected and transplanted in pots (upper caliber 24 cm, lower caliber 19 cm, height 26.5 cm). Before transplanting, the microorganisms were mixed well with the substrate, and the microorganisms were applied at the amount recommended by the manufacturer.

### 2.3 Indicator Measurement

#### 2.3.1 Determination of Physical and Chemical Properties of Substrates

Determination of substrate pH and conductivity: Natural air-dried substrate was mixed with pure water (1:5 v/v), stirred, and soaked for 2 hours. It was then filtered, and the pH and EC of the filtrate were measured with a pH meter and a conductivity meter, respectively.

Determination of organic carbon, alkaline dissolved nitrogen, and fast-acting phosphorus: The substrate's organic carbon, alkaline dissolved nitrogen, and fast-acting phosphorus contents were determined using the kit. The values were measured using a UV spectrophotometer. The operation procedures were carried out strictly according to the kit's instructions.

### 2.3.2 Determination of Tomato Plant Traits

(1) Plant height (cm): The height from the point of rhizome union to the end of growth was measured with a graduated tape measure.

(2) Stem thickness (cm): The thickness between the cotyledons and the first genuine leaf was measured with a vernier caliper.

(3) Leaf length and width (cm): Measure the height and width of the third genuine leaf with a graduated tape measure.

(4) Fresh weight (g): The whole plant was cleaned, dried, and weighed separately by electronic scale.

(5) Dry weight (g): Whole plants were put into envelopes and placed in the oven at 105 °C for 15 min. They were then dried at 80 °C until the weight remained unchanged and weighed by an electronic scale to obtain the dry weight.

(6) Relative chlorophyll content: Measured using a SPAD meter, selecting the 3rd fully expanded functional leaf below the growth point for measurement.

### 2.3.3 Determination of Tomato Fruit Traits

Fifteen tomato fruits from different plants were randomly selected from each treatment during the fruiting season to determine fruit indices.

(1) Fruit yield index:

1) Single fruit weight (g): Weigh the weight of a single fruit by electronic weighing.

2) Single-plant yield (kg): Leave six fruit spikes per plant and calculate the single-plant yield.

3) Fruit longitudinal and transverse diameter (cm): Use a ruler to measure the longitudinal and transverse diameter of the fruit.

(2) Fruit quality indicators: Fruit soluble solids content was measured using a handheld brix meter. Fruit soluble protein, soluble sugar, nitrate, and vitamin C content were calculated using the kit, and the operation steps were carried out strictly following the kit's instructions.

### 2.4 Data analysis

The results were averaged over three replications, analyzed by Excel software. The final result is presented in the form of "mean ± standard deviation".

## 3 Experimental results

### 3.1 Influence of microbial agents on nutrients in cultivation substrates

**Table 1 Effect of different microbial agents on the nutrients of cultivation substrates**

Treatment	Organic carbon (g/kg)	Alkaline nitrogen (g/kg)	Quick phosphorus (g/kg)	pH	EC(ms/cm)
CK	158.34±21.54 c	597.15±47.28 c	317.41±43.11 c	5.84±0.21 a	0.92±0.09 b
T1	205.81±18.22 a	857.17±33.76 a	517.97±31.45 a	6.34±0.55 a	1.08±0.11 a
T2	178.85±30.02 b	778.42±29.92 b	404.58±16.58 b	6.24±0.32 a	1.01±0.10 a
T3	168.72±17.45 c	642.43±41.01 c	431.62±33.62 b	6.02±0.23 a	0.99±0.07 b
T4	188.41±23.79 b	757.12±31.89 b	359.44±20.67 c	6.19±0.15 a	1.01±0.08 a
T5	164.23±11.28 c	609.12±25.66 c	471.01±17.92 b	6.24±0.11 a	0.98±0.06 b
T6	184.31±26.54 b	703.96±18.25 b	482.33±22.16 b	6.31±0.28 a	0.96±0.09 b

Note: Different letters in the table indicate significant differences below the 5% level.

This study first investigated the differences between nutrients in cultivation substrates under different microbial agent treatments to study the impact of microbial agents on tomato cultivation. Then, the physicochemical properties of the cultivation substrate, such as organic carbon content, alkaline dissolved nitrogen, quick-acting phosphorus, pH, and EC values, were measured.

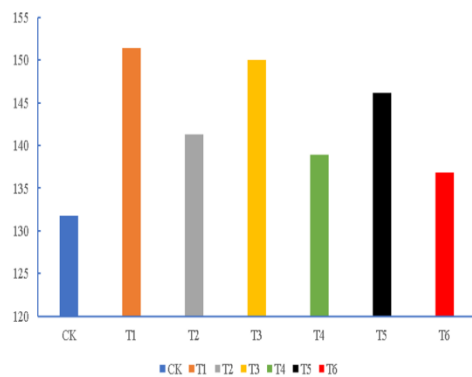
All the treatments with microbial additives significant-

ly increased the nutrients in the cultivation substrate compared to those without microbial additives (Table 1). Among them, the organic carbon content, alkaline dissolved nitrogen content, quick-acting phosphorus content, pH, and EC value of the cultivation substrate were the highest under T1 (*Bacillus subtilis*) treatment, which increased by 29.98, 43.54, 63.19, 8.56, and 17.39 %, respectively, compared with the control, indicating that the application of *Bacillus subtilis* could significantly increase the organic matter, alkaline dissolved nitrogen, quick-act-

ing phosphorus content, and salt ion concentration. Combined, microbial agents can increase the nutrients in the cultivation substrate and provide the required nutrients for crop growth and development. Among them, T1 (*Bacillus subtilis*) best affects organic matter decomposition, nitrogen fixation, and phosphorus solubilization and can be used as a cultivation substrate additive to release nutrients.

### 3.2 Effect of microbial fungicides on the growth of tomato plants under substrate cultivation

Microbicides increase the nutrient content in the cultiva-



**Fig. 1** Effect on the height of tomato plants

Plant height is one of the commonly used visual growth indicators. Based on the investigation, it was found that all treatments increased the plant height of tomato plants compared to no microbial agent (131.74 cm) (Fig. 1). In addition, among the microbial treatments, the plant height of tomato plants in the cultivated substrate under T1 (*Bacillus subtilis*) treatment was the largest at 151.43 cm, followed by T3 (150.04 cm), T5 (146.16 cm), T2 (141.27 cm), T4 (138.89 cm), and the smallest at T6 (136.82 cm). The data were analyzed. The research results showed that the height of plants was significantly better after treatment with T1, T2, T3, and T5, indicating that the application of microbial agents such as T1 (*Bacillus subtilis*), T2 (*Bacillus cereus*), T3 (*Penicillin*), and T5 (*Bacillus suis*) could significantly increase the height of the crop plants.

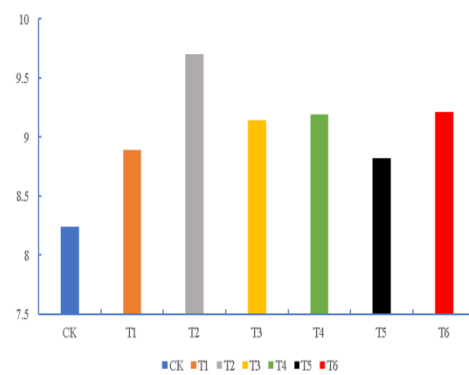
The microbial agents were able to increase the height of tomato plants in the cultivation substrate and promote plant growth. T1 (*Bacillus subtilis*) had the best influence on tomato plant height promotion, followed by T3 (*Penicillin*), T5 (*Bacillus suis*), and T2 (*Bacillus cereus*).

#### 3.2.2 Effect of microbial fungicides on stem thickness of tomato under substrate cultivation

The stem is the main organ of crop growth, and stem

tion substrate, and the crop will grow and develop better after absorption. In this study, we measured the traits of tomato plants, such as plant height, leaf length, stem thickness, leaf width, fresh weight, dry weight, and relative chlorophyll content, to investigate the effects of different microbial preparations on the growth and development of tomato plants under different cultivation substrate treatments.

#### 3.2.1 Effect of microbial fungicides on tomato plant height under substrate cultivation

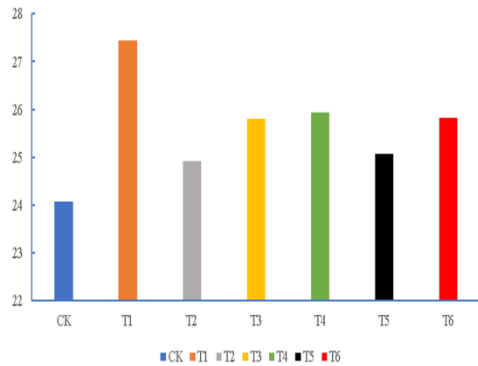


**Fig. 2** Effect on stem thickness of tomato

thickness can directly determine the plant's ability to transport nutrients and the support capacity of the above-ground part. Based on the investigation, it was found that all treatments increased the stem thickness of tomato plants compared to no microbial agent (8.24 mm) (Fig. 2). In addition, among the microbial agent treatments, the stem thickness of tomato plants in cultivation substrate under T2 (*Bacillus cereus*) treatment was the largest at 9.70 mm, followed by T6 (9.21 mm), T4 (9.19 mm), T3 (9.14 mm), T1 (8.89 mm), and the smallest was T5 (8.82 mm). From the results, it can be seen that the plants treated with T2, T3, T4, and T6 were significantly higher than those treated with other treatments, indicating that the application of microbial fungicides such as T2 (*Bacillus cereus*), T3 (*Penicillin*), T4 (*Xylomycetes*), and T6 (*Bacillus subtilis* (botany)) could significantly increase the stem thickness of the crop.

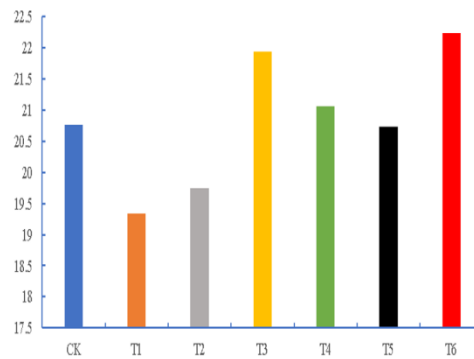
The microbial agents could improve the stem thickness of tomato plants in the cultivation substrate, and promote the transportation of nutrients between the above-ground leaves and the below-ground root system, thereby promoting the growth of the aboveground part of the plant.

#### 3.2.3 Effect of microbial fungicides on leaf length and width of tomato under substrate cultivation



**Fig. 3 Effect on leaf length of tomato**

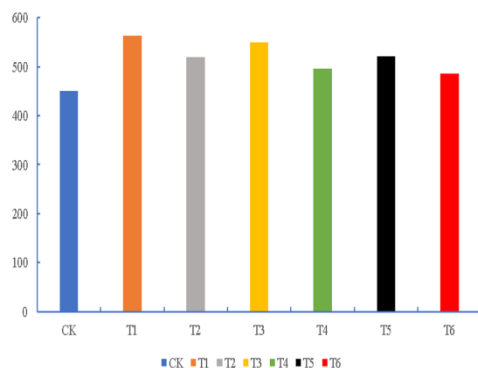
Leaves are essential organs for photosynthesis and nutrient conversion in crops. Therefore, we investigated leaf length and width. The results showed that all treatments increased the leaf length of tomato plants compared with no microbial additives (Fig. 3). Still, the influence on the leaf width of tomato plants was not very obvious (Fig. 4). Moreover, in terms of leaf length, the maximum leaf length of tomato plants in the cultivated substrate under T1 (*Bacillus subtilis*) treatment of microbial agents was found to be 27.43 cm, followed by T4 (25.94 cm), T6 (25.83 cm), T3 (25.80 cm), and T5 (25.07 cm). In contrast, the minimum was found in T2, which was 24.92



**Fig. 4 Effect on leaf width of tomato**

cm. In terms of leaf width, only T6, T3, and T4 treatments were higher than CK in the cultivation substrate, indicating that the application of microbial fungicides such as T6 (*Bacillus subtilis* (botany)), T3 (Penicillin), and T4 (*Xylomyces*) could increase the leaf width of the crop. The microbial agents could improve the leaf length of tomato plants in the cultivation substrate and promote the elongation and growth of the leaves, thus increasing the intensity of photosynthesis and promoting the accumulation of substances.

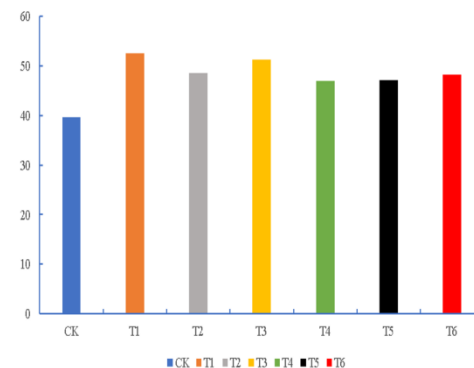
**3.2.4 Effect of microbial agents on the fresh and dry weight of tomato under substrate cultivation**



**Fig. 5 Effect on fresh weight of tomato plants**

Fresh weight and dry weight can show the accumulation of material after absorbing water and nutrients, which is one of the critical indicators of crop growth. Therefore, we measured the fresh and dry weight of tomato plants. The results showed that all treatments increased tomato plants' fresh and dry weights (450.42 g, 39.69 g) compared to no microbial additives (Figures 5 and 6).

Moreover, in terms of fresh weight, the maximum fresh weight of tomato plants in the cultivated substrate under T1 (*Bacillus subtilis*) treatment of microbial agents was 562.75 g, followed by T3 (550.01 g), T5 (521.39 g), T2 (518.77 g), T4 (496.22 g). In contrast, the minimum weight was recorded in T6 (486.17 g). In terms of dry

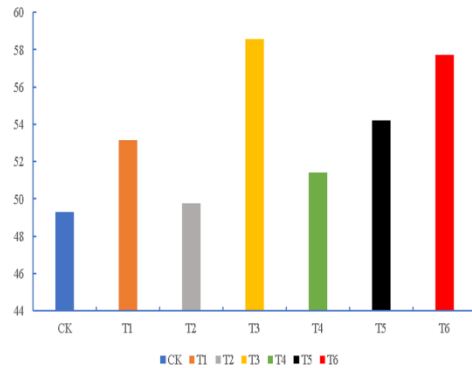


**Fig. 6 Effect on the dry weight of tomato plants**

weight, adding microbial agent treatments increased the dry weight of tomato plants in the cultivated substrate. The fresh weight of tomato plants in the cultivated substrate under microbial agent treatment T1 (*Bacillus subtilis*) was the largest, 52.47 g, followed by T3 (51.33 g), T2 (48.52 g), T6 (48.17 g), T5 (47.21 g), and T4 was the smallest at 46.98 g. From the results, Data analysis showed that applying microbial additives could dramatically increase the fresh and dry weights of the crop plants, promote water and nutrient uptake by the plants, and improve the accumulation of material. In terms of the strength of the effect, T1 (*Bacillus subtilis*) showed the best promotion of tomato plants' fresh and dry weights, followed by T3

(Penicillin).

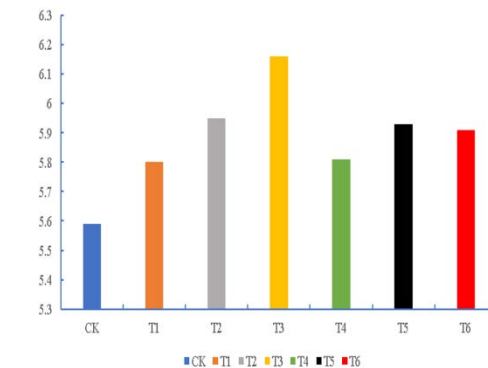
### 3.2.5 Effect of microbial fungicides on the relative chlorophyll content of tomato



**Fig. 7 Effect on chlorophyll in tomato** **Fig. 8 Effect on the longitudinal diameter of tomato fruits**

Chlorophyll is the green pigment in plant cells that allows photosynthesis. Its content is one of the most critical indicators of leaf maturity and nutritional status. We measured the relative chlorophyll content of tomato plant leaves in vivo using a handheld chlorophyll meter. The results showed that all treatments increased the relative chlorophyll content of tomato leaves compared to no microbial agent (49.28 SPAD) (Fig. 7). Moreover, among the microbial agent treatments, the relative chlorophyll content of tomato leaves in the cultivated substrate under T3 (Penicillin) treatment was the largest at 58.56 SPAD, followed by T6 (57.72 SPAD), T5 (54.21 SPAD), T1 (53.17 SPAD), and T4 (51.42 SPAD), whereas T2 was the smallest at 49.76 SPAD. Data analysis revealed that the relative chlorophyll content of tomato leaves in the cultivated substrate under T1, T3, T4, T5, and T6 treatments was significantly higher than the rest of the treatments, indicating that the application of microbial agents such as T1 (*Bacillus subtilis*), T3 (Penicillin), T4 (*Xylomyces*), T5 (*Bacillus suis*), and T6 (*Bacillus subtilis* (botany)) could significantly increase the relative chlorophyll content.

### 3.3 Effect of microbial agents on tomato fruits under substrate cultivation

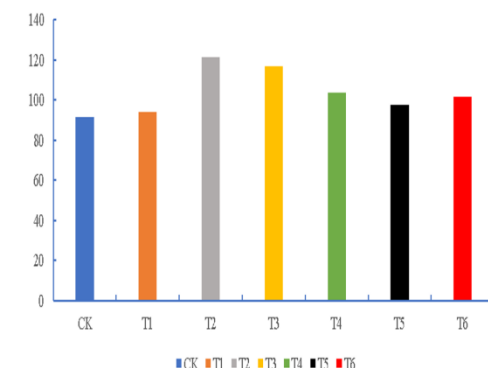
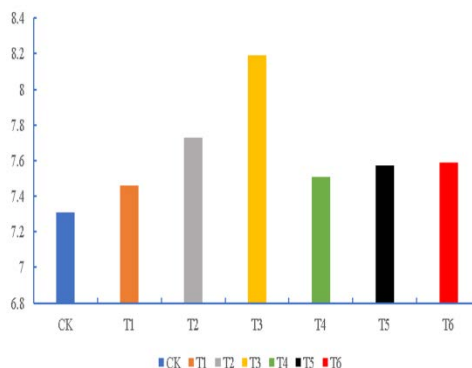


Overall, the use of microbial fungicides effectively increased the relative chlorophyll content of tomato leaves in the cultivation substrate, which effectively promoted photosynthesis in tomato plant leaves. Stronger photosynthesis will help with plant material accumulation and growth.

### 3.3 Effect of microbial agents on tomato fruits under substrate cultivation

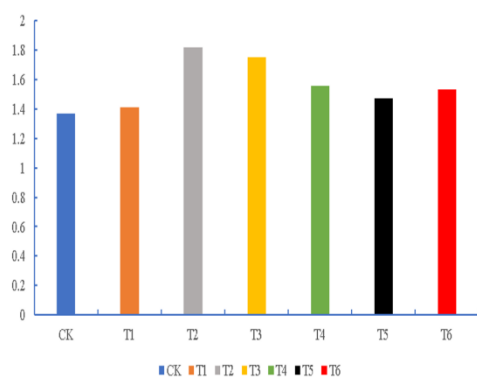
Under the action of microbial agents, the growth and development of tomato plants will be better, which will lay a solid foundation for fruit development and ripening. In this study, the tomato fruit traits such as transverse diameter, longitudinal diameter, single fruit weight, single plant yield, soluble solids content, soluble protein content, soluble sugar content, nitrate content, vitamin C content, and other indexes were measured under different microbial agent treatments.

#### 3.3.1 Effect of microbial fungicides on the longitudinal and transverse diameter of tomato fruits under substrate cultivation



**Fig. 9 Effect on the transverse diameter** **Fig. 10 Effect on single fruit weight**

Fruit longitudinal and transverse diameters are two of the most critical indicators of fruit size and are the most commonly used and intuitive measurements. Our measurements of ripened tomato fruits' longitudinal and transverse diameters revealed that all treatments increased the diameters compared to no microbicides (5.59 cm, 7.31 cm) (Figs. 8 and 9). Moreover, in terms of the longitudinal diameter of fruits, the microbial agent treatment T3 (Penicillin) had the largest longitudinal diameter of tomato fruits in the cultivated substrate under the treatment of T3 (Penicillin) at 6.16 cm, followed by T2 (5.95 cm), T5 (5.93 cm), T6 (5.91 cm), and T4 (5.81 cm). In contrast, the smallest of the microbial agent treatments was T1 (Bacillus subtilis) at 5.80 cm. The data were analyzed, and it

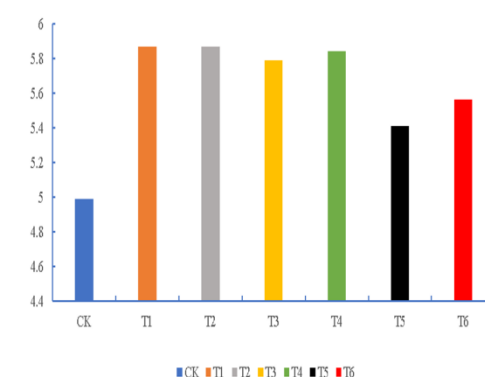


**Fig. 11 Effect on yield of tomato monocultures**

We measured the individual fruit weight of ripened tomato fruits and the fruit weight per plant. It was found that all treatments increased the individual fruit weight and individual plant yield of tomatoes compared to no microbial additives (91.42 g, 1.37 kg) (Figs. 10 and 11). Moreover, in terms of single fruit weight of tomato, T2 (Bacillus cereus) treatment showed the highest single fruit weight of 121.42 g, followed by T3 (116.54 g), T4 (103.72 g), T6 (101.72 g), T5 (97.72 g), while T1 was the smallest with 91.42 g. The weight of the tomato fruits was also higher than the others. The Analysis of the data revealed that the cultivated fruits of tomato in the substrate under the treatments of T2 and T3 had the highest single fruit weight and yield per plant (Fig. 10 and Fig. 11). The single fruit weight of tomato fruits in the substrate was significantly higher than the rest of the treatments, indicating that the application of T2 (Bacillus cereus) and T3 (Penicillin) microflora could dramatically increase the single fruit weight of tomato. Regarding tomato yield per plant, tomato yield per plant in the cultivated substrate under T2 treatment was the largest at 1.82 kg. The yield of tomato plant per plant in the cultivated substrate under the T2 and T3 treatments was significantly higher than that in the rest of the treatments, which indicated that the application of

was found that The longitudinal diameter of tomato fruits in the cultivated substrate under T1 to T6 treatments was significantly higher than that of the control. There was no significant difference between the treatments, indicating that applying microbial agents could dramatically increase the longitudinal diameter of the fruits. Regarding transverse diameter, tomato fruits in the cultivated substrate under T3 treatment had the largest transverse diameter of 8.19 cm.

### 3.3.2 Effect of microbial fungicides on fruit weight per fruit and yield per plant of tomato fruits under substrate cultivation



**Fig. 12 Effect on the soluble solids content**

T2 (Bacillus cereus) and T3 (Penicillin) microbials could dramatically increase the fruit weight per plant. In conclusion, microbial agents can increase tomatoes' single fruit weight and single plant yield in the cultivation substrate, promote the accumulation of fruit matter, and improve fruit yield. Among them, T2 (Bacillus cereus) showed the best promotion of tomato fruit weight per fruit and yield per plant, followed by T3 (Penicillin) and T4 (Xylomyces).

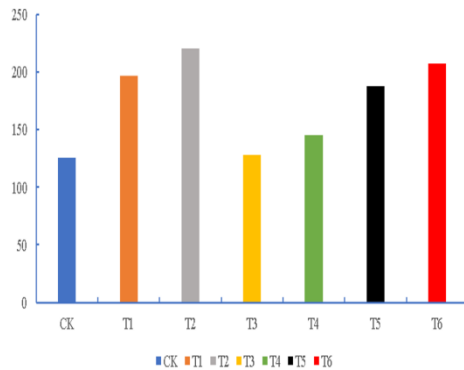
### 3.3.3 Effect of microbial agents on the soluble solids content of tomato fruits under substrate cultivation

Soluble solids content is an essential indicator of the content of soluble saccharides or other soluble substances and one of the most critical indicators of fruit quality. We measured the soluble solids content of tomato fruits with a handheld brix meter. We found that all treatments increased the soluble solids content in tomato fruits compared to no microbial agent (4.99%) (Fig. 12). Moreover, among the microbial agent treatments, the soluble solids content of tomato fruits was maximum under T1 (Bacillus subtilis) and T2 (Bacillus cereus) treatments, both of which were 5.87 %, followed by T4 (5.84 %), T3 (5.79 %), and T6 (5.56 %), whereas T5 was the smallest at 5.41

%. Data analysis revealed that the soluble solids content of tomato fruits in the cultivation substrate under T1 to T6 treatments was significantly higher than the rest of the treatments, indicating that applying microbial agents could dramatically increase the tomato fruits' soluble solids content. In summary, the microbial agents were able to

increase the soluble solids content of tomato fruits in the cultivation substrate, promote the accumulation of substances, and improve the taste of fruits.

### 3.3.4 Effect of microbial agents on vitamin C content of tomato fruits under substrate cultivation

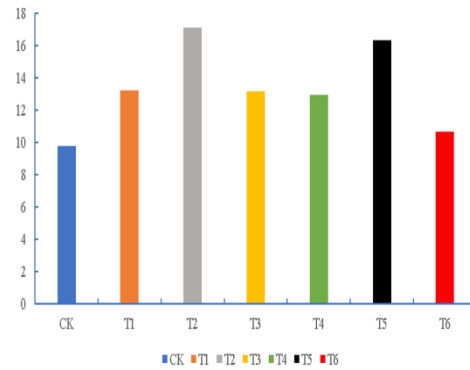


**Fig. 13 Effect on vitamin C content**

Vitamin C content in fruits is one of the most critical indicators of fruit quality. We determined the vitamin C content of tomato fruits. We found that all treatments increased the vitamin C content of tomato fruits compared to no microbial agent (125.82 µg/g) (Fig. 13). Moreover, among the microbial treatments, vitamin C content of tomato fruits under T2 (*Bacillus cereus*) treatment was maximum at 220.81 µg/g, followed by T6 (207.19 µg/g), T1 (196.58 µg/g), T5 (187.68 µg/g), T4 (145.17 µg/g), while T3 was minimum at 128.38 µg/g. Data analysis revealed that the vitamin C content of tomato fruits in the cultivation substrate under T2 and T6 treatments was significantly higher than the rest of the treatments, indicating that the application of microbial fungicides such as T2 (*Bacillus cereus*) and T6 (*Bacillus subtilis* (botany)) could significantly increase the vitamin C content of tomato fruits.

### 3.3.5 Effect of microbial agents on the soluble protein content of tomato fruits under substrate cultivation

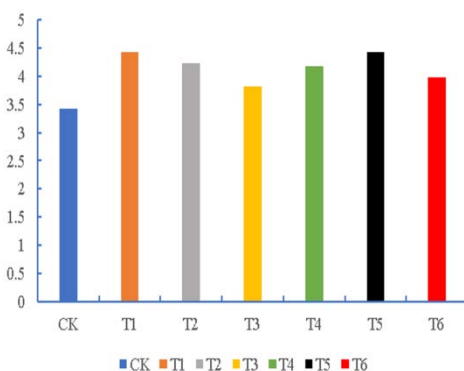
Soluble protein is an essential osmoregulatory substance



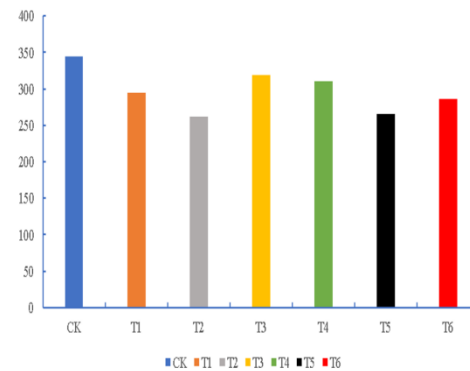
**Fig. 14 Effect on the soluble protein content**

and nutrient, and fruit soluble protein content is one of the critical indicators of fruit quality. We determined the soluble protein content of tomato fruits. We found that all treatments increased the soluble protein content of tomato fruits compared to no microbial agent (9.79 µg/g) (Fig. 14). Moreover, among the microbial treatments, the soluble protein content of tomato fruits under T2 (*Bacillus cereus*) treatment was maximum at 17.13 µg/g, followed by T5 (16.32 µg/g), T1 (13.22 µg/g), T3 (13.18 µg/g), T4 (12.95 µg/g), whereas T6 was minimum at 10.68 µg/g. Analysis of the data It was found that the soluble protein content of tomato fruits in the cultivation substrate under T2 and T5 treatments was significantly higher than the rest of the treatments, indicating that the application of T2 (*Bacillus cereus*) and T5 (*Bacillus subtilis*) microbial fungicides could dramatically increase the soluble protein content of tomato fruits.

### 3.3.6 Effect of microbial fungicides on the soluble sugar content of tomato fruits under substrate cultivation



**Fig. 15 Effect on the soluble sugar content**



**Fig. 16 Effect of on nitrate content**



Soluble sugars are monosaccharides and polysaccharides that can be dissolved in water to produce a sweet flavor, and fruit soluble sugar content is one of the most critical indicators of fruit quality. We determined the soluble sugar content of tomato fruits. We found that all treatments increased the soluble sugar content in tomato fruits compared to no microbial agent (3.42 %) (Fig. 15). Moreover, among the microbial agent treatments, the soluble sugar content of tomato fruits under T1 (*Bacillus subtilis*) treatment was maximum at 4.43 %, followed by T5 (4.42 %), T2 (4.23 %), T4 (4.18 %), T6 (3.98 %), while T3 was minimum at 3.82 %. Data analysis revealed that the soluble sugar content of tomato fruits in the cultivation substrate under T1, T2, T4, and T5 treatments was significantly higher than the rest of the treatments, indicating that the application of microbial agents such as T1 (*Bacillus cereus*), T2 (*Bacillus cereus*), T4 (*Xylococcus hartshorn*), and T5 (*Bacillus subtilis*) could significantly increase the soluble sugar content of tomato fruits. Taken together, the microbial agents increased the soluble sugar content of tomato fruits in the cultivation substrate and improved their nutritional quality. T1 (*Bacillus subtilis*) had the best impact, followed by T2 (*Bacillus cereus*).

### 3.3.7 Effect of microbial agents on nitrate content of tomato fruits under substrate cultivation

Nitrate in crop leaves or fruits can be converted to nitrite after being absorbed by the human body, and nitrite is a potent carcinogen that can threaten human health. Therefore, the nitrate content in the fruit is also one of the indicators of fruit quality. We determined the nitrate content of tomato fruits. We found that all treatments reduced the nitrate content of tomato fruits compared to no microbial agent (344.47  $\mu\text{g/g}$ ) (Fig. 16). Moreover, among the microbial treatments, the minor nitrate content of tomato fruits under T2 (*Bacillus cereus*) treatment was 262.47  $\mu\text{g/g}$ , followed by T5 (265.86  $\mu\text{g/g}$ ), T6 (285.70  $\mu\text{g/g}$ ), T1 (294.22  $\mu\text{g/g}$ ), T4 (310.52  $\mu\text{g/g}$ ), and the smallest was T3 (318.71  $\mu\text{g/g}$ ). Data analysis revealed that the nitrate content of tomato fruits in the cultivation substrate under T2 and T5 treatments was significantly lower than the rest of the treatments, indicating that the application of microbial agents T2 (*Bacillus cereus*) and T5 (*Bacillus suis*) could dramatically reduce the nitrate content of tomato fruits.

## 4 Discussion and conclusions

This study compared the differences between the nutrients in the cultivation substrate under different microbial agent treatments and found that the addition of microbial agents was able to increase the nutrients in the cultivation substrate to a certain extent, which indicated that the mi-

crobial agents had a specific effect of nitrogen fixation, phosphorus solubilization, and decomposition of organic matter. Applying *Bacillus subtilis* can significantly increase the content of organic matter, alkali-dissolved nitrogen, quick-acting phosphorus, and salt ion concentration in the cultivation substrate. It can be used as a microbial bacterial agent for accelerating the decomposition of the nutrients in the cultivation substrate, which is in line with the results of the research report that *Bacillus subtilis* has the impact of fixing nitrogen, dissolving phosphorus, and solubilizing potassium, and improves the uptake of nutrients in crops, which leads to better growth of plants<sup>[21]</sup>.

In this study, we compared the traits of tomato plants in the cultivation substrate under different microbial agent treatments. Applying *Bacillus subtilis* could significantly increase the plant height, leaf length, fresh weight, and dry weight of tomato plants in the cultivation substrate. Applying *Bacillus cereus* and *Bacillus subtilis* (botany) could significantly increase tomato plants' stem thickness and leaf width in the cultivation substrate. In addition, this study also compared the differences between tomato fruit traits in the cultivation substrate under different microbial agent treatments and found that the addition of microbial agent treatments was able to improve tomato fruit traits such as transverse diameter, longitudinal diameter, fruit weight per unit, yield per unit, as well as the content of soluble solids, vitamin C, soluble protein, soluble sugar, nitrate, etc., to a certain degree in the tomato plants in the cultivation substrate, etc. Among them, the application of *Bacillus cereus* can promote the transformation of internal substances in tomato fruits, make tomato fruits develop better, improve the indexes of tomato fruits, and reduce nitrate content. While the application of Penicillin can significantly increase the longitudinal and transverse diameters of tomato fruits in the cultivation substrate, the application of *Bacillus subtilis* can substantially increase the content of soluble solids and soluble sugars.

The research results show that introducing microbial agents not only optimizes the physical and chemical properties of the cultivation substrate but also significantly increases the nutrient content in the plant body, promotes root development, and ultimately improves tomato yield and quality. Among the six microbial preparations tested, *Bacillus subtilis* and *Bacillus cereus* showed the most outstanding effects, with extremely positive effects on the growth and development of tomato plants. This discovery is of great significance for promoting sustainable agricultural development. Traditional agriculture often relies on a large amount of fertilizers and pesticides, which not only increases production costs but may also cause pollution to the environment, affecting soil health and ecological balance. As a type of biological fertilizer, microbial agents

can reduce the input of chemicals while improving crop yield and quality, which is an important way to achieve green and organic agriculture. Specifically, applying *Bacillus subtilis* and *Bacillus cereus* provides an efficient and high-quality solution for tomato substrate cultivation. Therefore, in future agricultural production, these two microbial agents can be further studied and promoted to ensure food safety, improve the quality of agricultural products, and achieve sustainable development of agricultural production. In summary, this study reveals the positive role of microbial agents in tomato cultivation and provides valuable reference and inspiration for efficient and high-quality tomato substrate cultivation. With the continuous deepening of research on microbial agents, it is believed that more similar biological fertilizers will be developed in the future, contributing to the green development of agricultural production.

## References

- [1] Liang J P, Xue Z Q, Yang Z Y, et al. Effects of microbial organic fertilizers on *Astragalus Membranaceus* growth and rhizosphere microbial community[J]. *Annals of Microbiology*, 2021, 71(1).
- [2] Gupta V V S R, Germida J J. Distribution of microbial biomass and its activity in different soil aggregate size classes affected by cultivation[J]. *Soil Biology and Biochemistry*, 1988, 20 (6): 777-786.
- [3] Crowley D E. Iron Nutrition in Plants and Rhizospheric Microorganisms: Microbial siderophores in the plant rhizosphere[J]. Springer Netherlands, 2006, 8: 169-198.
- [4] Egamberdieva D, Kucharova Z, Davranov K, et al. Bacteria can control foot and root rot and promote cucumber growth in salinated soils[J]. *Biology and Fertility of Soils*, 2011, 47 (2): 197-205.
- [5] Mohite B. Isolation and characterization of indole acetic acid (IAA) producing bacteria from rhizospheric soil and its influence on plant growth[J]. *Journal of Soil Science and Plant Nutrition*, 2013, 13:638-649.
- [6] Zhang N, Wang D D, Liu Y P, et al. Effects of different plant root exudates and their organic acid components on chemotaxis, biofilm formation, and colonization by beneficial rhizosphere-associated bacterial strains[J]. *Plant Soil*, 2014, 374: 689-700.
- [7] Pineda A, Zheng S, Loon V J J, et al. Helping plants to deal with insects: the role of beneficial soil-borne microbes[J]. *Trends in Plant Science*, 2010, 15 (9): 507-514.
- [8] Li Y L, Guo Q, Li Y Z, Sun Y F, et al. *Streptomyces* (genus of fungi) Act12 controls tomato yellow leaf curl virus disease and alters rhizosphere microbial communities[J]. *Biology and Fertility of Soils*, 2019, 55: 149-169.
- [9] Li H Y, Qiu Y Z, Yao T, et al. Effects of PGPR microbial inoculants on the growth and soil properties of *Avena sativa*, *Medicago sativa*, and *Cucumis sativus* seedlings[J]. *Soil and Tillage Research*, 2020, 199: 104577.
- [10] Ryu M H, Zhang J, Toth T, et al. Control of nitrogen fixation in bacteria that associate with cereals[J]. *Nature Microbiology*, 2020, 5(2): 314-330.
- [11] Wang T, Liu M Q, Li H X. Inoculation of phosphate-solubilizing bacteria *Bacillus subtilis* B1 increases available phosphorus and growth of peanut in acidic soil[J]. *Acta Agriculturae Scandinavica, Section B-Soil & Plant Science*, 2014, 64(3): 252-259.
- [12] Borah A, Das R, Mazumdar R, et al. Culturable endophytic bacteria of *Camellia* species endowed with plant growth-promoting characteristics[J]. *Journal of Applied Microbiology*, 2019, 127 (3): 825-844.
- [13] Rashid M I, Mujawar L H, Shahzad T, et al. Bacteria and fungi can contribute to nutrient bioavailability and aggregate formation in degraded soils[J]. *Microbiological Research*, 2016, 183: 26-41.
- [14] Torres M, Llamas I, Torres B, et al. Growth promotion on horticultural crops and antifungal activity of *Bacillus XT1*[J]. *Applied Soil Ecology*, 2020, 150: 103453.
- [15] Jiang YJ, Liu YS, Zhang XY, et al. Biofilm application in the microbial biochemicals production process[J]. *Biotechnology Advances*, 2021, 48: 107724.
- [16] Zhao YN, Zhang MS, Yang W, et al. Effects of microbial inoculants on phosphorus and potassium availability, bacterial community composition, and chili pepper growth in a calcareous soil: a greenhouse study[J]. *Journal of Soils and Sediments*, 2019, 19: 3597-3607.
- [17] Ahmad F, Ahmad I, Khan M K. Screening of free-living rhizospheric bacteria for their multiple plant growth promoting activities[J]. *Microbiological research*, 2008, 163 (2): 173-81.
- [18] Kilian M, Steiner U, Krebs B, et al. FZB24 *Bacillus subtilis*-Mode of action of a microbial agent enhancing plant vitality[J]. *Pflanzenschutz-Nachrichten Bayer*, 2000, 53 (1): 72-93.