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# Physiological impact of *Trichoderma viride* agents on the quality and production of melon that is grown on soils continuously cropped to melon

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The issue of ongoing cropping barriers is getting worse as China's melon planting area steadily grows, and the melon industry's sustainable growth is being negatively impacted by the steadily diminishing yield and quality of the fruit. *Trichoderma* is a probiotic that can enhance the physiological traits of crops, encourage their growth, and raise their yield and quality. It is yet unknown, though, how *Trichoderma* influences the growth, physiological traits, and yield of melon grown on soils continuously cropped to melon. *Trichoderma viride* kf57 agents at  $1.0 \times 10^4$ ,  $8.0 \times 10^4$ ,  $6.4 \times 10^5$ , and  $5.12 \times 10^6$  CFU/g and no *Trichoderma viride* agents (CK) were utilized as treatments. At the seedling and fruiting stages, a pot experiment and a bedding experiment were conducted to study the physiological properties and yield of melon under varying concentration of *Trichoderma viride* kf57 agents. As a result of the application of *T. viride* agents, the indexes of melon seedlings were all significantly improved. The treatment of  $6.4 \times 10^5$  CFU/g had the best promoting effect on the morphology of melon seedlings, and the plant height, stem diameter, leaf area, fresh weight of whole plant, dry weight of whole plant, root shoot ratio, and strong seedling index of melon seedlings increased by 90.39, 46.30, 37.55, 81.35, 100.62, 51.47, and 240.00%, respectively, compared with CK. The results showed that different amounts of *T. viride* agents could improve physiological and biochemical indices of melon leaves during the fruiting stage; the treatment of  $6.4 \times 10^5$  CFU/g was the most effective; chlorophyll content, nitrate nitrogen content, sucrose content, reducing sugar content, free proline content, nitrate reductase (NR) activity, peroxidase (POD) activity, and superoxide dismutase (SOD) activity of melon leaves increased at 30 days after melon pollination. Melon quality and yield was also enhanced by the use of *T. viride* agents, with the treatment of  $6.4 \times 10^5$  CFU/g *T. viride* agents having the best boosting effects. The melon fruit's transverse diameter, vertical diameter, single fruit weight, and yield all increased. The amount of soluble solids, vitamin C, soluble protein, soluble sugar, and sugar acid ratio also increased. In conclusion, by promoting the morphology of melon grown on soils continuously cropped to melon seedlings, *T. viride* agents can improve the physiological characteristics of melon grown on soils continuously cropped to melon and improve the production and quality qualities of melon. When using *T. viride* kf57 agents,  $6.4 \times 10^5$  CFU/g is the highest effective dosage. The study revealed that *T. viride* agents had significant potential as biological agents as they showed good results in melon yield and quality formation, as well as in enhancing seedling quality.

## KEYWORDS

continuously cropped, melon, physiological and biochemical indices, quality, *Trichoderma viride* agents, yield

## 1 Introduction

Melon (*Cucumis melo* L.) is ranked among the top 10 fruit vegetables in the world. Melon's rich flavor and crispy texture make it a popular choice among customers. It has a lengthy history of cultivation and is commonly cultivated worldwide (Grumet et al., 2021). China is an important producer and consumer of cantaloupe in the world (Zhang J. et al., 2019). According to data from the Food and Agriculture Organization of the United Nations (FAO), in 2021, the planting area of cantaloupe in China was 387,000 hectares, with a yield of  $1.41 \times 10^6$  kg, accounting for 35.9 and 49.2% of the world's cantaloupe cultivation area and yield, respectively. The unit area yield of sweet melons in China has increased from  $3.57 \times 10^8$  kg/hm<sup>2</sup> in 2020 to  $3.64 \times 10^8$  kg/hm<sup>2</sup> in 2021. This indicates that China's melon farming has significant competitive advantages, growth potential, and great economic value. China's melon production and planting area have consistently ranked top in the world in recent years (Cai et al., 2023) due to the ongoing advancements in melon growing technologies. Simultaneously, persistent cropping barriers in the industrialization and intensive melon cultivation process are getting more serious, which eventually results in a decline in melon quality and yield. This has become a significant factor limiting farmers' income and adversely affecting the long-term growth of China's melon industry (Ku et al., 2022). The demand for superior cantaloupe is rising in tandem with the expansion of the Chinese economy and the ongoing improvement in the standard of living of the people. In the present melon production cycle, improving melon quality and yield has emerged as a key research area (Cui et al., 2022). In actual production, different agricultural management techniques such as soil treatment, sensible rotation, soil sterilization, and artificial introduction of beneficial microorganisms have become the main means of improving melon yield and quality (Tang et al., 2023) due to the diversity and uneven quality of melon varieties, as well as differences in storage and transportation and disease resistance. One of the primary methods of improving soil microbial environment and growth of plant is the application of *Trichoderma* spp.

*Trichoderma* is one of the most researched and utilized of all the biocontrol fungi (Srivastava et al., 2012). *Trichoderma* has a positive regulatory effect on the morphology, physiology, nutrient dissolution and absorption, yield growth, and induced disease resistance of plants by speeding up their growth and development, increasing their absorption of nutrients, improving their tolerance to biotic and abiotic stress, and improving the rhizosphere environment. Disease resistance is induced, stress resistance is reinforced, and plant development is promoted (Sood et al., 2020).

There are currently just a few studies on the use of *Trichoderma* in melon, with the majority of the studies concentrating on the plant antioxidant defense system, soil microbial amount, soil enzyme activity, and the influence of certain agronomic parameters as well as the biological control effect of melon wilt. For instance, four *Trichoderma* species (*T. viride*, *T. harzianum*, *T. helicum*, and *T. erinaceum*) were investigated by Boughalleb-M'Hamdi et al.

(2018), who also assessed the species' efficacy in controlling soilborne fungi in melon and watermelon. According to the findings, *Trichoderma* not only lessened the severity of blight but also enhanced the melon and watermelon's growth metrics and the rate at which their shoot and root dry weights developed. According to Bernal-Vicente et al. (2015), melon plants' antioxidant defense system was regulated by inoculation with *T. harzianum* T-78, which also increased the activity of peroxidase and the ascorbic acid cycle enzymes. These findings suggest that *T. harzianum* may boost melon plant yield. *T. harzianum* treatment of melon seeds enhanced soil microbial biomass and enzyme activity during the flowering stage, leading to increase in root length, indicating a positive influence on melon root development, according to Galletti et al. (2015).

Nonetheless, more studies on *Trichoderma* on different plants, as those conducted by Ahmad et al. (2015), have demonstrated that *Trichoderma* is a significant plant growth-promoting rhizosphere fungus (PGPF) that has the ability to stimulate plant growth. For instance, Zhao et al. (2021) discovered that *T. afroharzianum* TM2-4 culture filtrate can create bioactive compounds and aid in tomato seed germination. They also discovered that tomato hypocotyl length, root length, and vitality index increased after 100 times dilution. According to Yani et al. (2019), *Trichoderma* spp. treatment raised the plant height, root length, and tuber weight of garlic plants. *Trichoderma viride* and Arbuscular mycorrhizal fungi were shown by Metwally and Al-Amri (2020) to be able to increase the fresh weight and dry weight of onions as well as morphological indices including leaf area, stem length, and root length as well as the pigment content of onions. Numerous studies have demonstrated that *Trichoderma* can enhance the physiological and biochemical traits of plants, which results in an increase in production and an improvement in quality. As an illustration, Li et al. (2022) discovered that *Trichoderma* considerably raised *Mongholicus Astragalus*'s root biomass, root length, nitrate reductase, and soil urease activity. *Trichoderma* agents were discovered by Kuang et al. (2024) to boost the activity of SOD (superoxide dismutase), POD (peroxidase), CAT (catalase), PPO (polyphenoloxidase), and PAL (Phenylalanineammonialyase) in chrysanthemum leaves. The fresh weight above ground, fresh weight below ground, root to shoot ratio, root vitality, and plant height all experienced increases. Pro (Proline), MDA (malondialdehyd), and total phenolic content of rice seeds treated with *Trichoderma asperelloides* and *Trichoderma brevicompactum* were shown to be significantly different from those of untreated plants by Quazi et al. (2024). According to Vultaggio et al. (2024), inoculating with *T. atroviride* considerably enhanced the commercial production of strawberries, total sugar, and anthocyanin content when compared to untreated plants. According to Lian et al. (2023), a formulation of *Trichoderma harzianum* agents containing  $10^6$  cfu/g also enhanced the quality index and yield production of cucumbers. In contrast to CK1 ( $10^4$  cfu/g *Fusarium oxysporum* powder only), the fruit of cucumbers had higher levels of soluble sugar, vitamin C, soluble protein, and soluble solids and an increase in yield.

*Trichoderma* products have been used all around the world for more than 60 years. *Trichoderma* can produce mycelium, conidia,

and chlamyospores during the course of its life cycle. At present, there are more than 50 kinds of commercial *Trichoderma* preparations at home and abroad (Woo et al., 2006), such as *Trichoderma harzianum* T22 strain in the United States and *Trichoderma harzianum* T39 strain in Israel; *Trichoderma* preparations Trichodry and Trichoflow in New Zealand; *Trichoderma* preparations Myc01 in Russia; *Trichoderma* YC458 from South Korea; and the mixed biocontrol agents TUSAL of *Trichoderma harzianum* and *Trichoderma viridis* in Spain (Sanchez et al., 2019). Therefore, creating stable *Trichoderma* conidium preparations is crucial for producing plants with high yields and good quality. In this study, the *Trichoderma viride* kf57 conidium agents was used to systematically investigate its physiological effects on the seedling morphogenesis, yield, and quality formation of melon grown on soils continuously cropped to melon with the aim of providing technical assistance for promoting the quality and secure production of melon and providing a theoretical foundation development and promotion of *Trichoderma* agents.

## 2 Materials and methods

### 2.1 *Trichoderma* strain characteristics

The *Trichoderma* research team from the Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, China, contributed the tested strain of *Trichoderma viride* kf57.

From the rhizosphere soil of pine trees in Heilongjiang, China, strain *T. viride* kf57 was isolated, identified, and stored at the *Trichoderma* Research Group of the Institute of Plant Protection, Chinese Academy of Agricultural Sciences. HY1-22 was the strain number at first. The results of the plate confrontation test shown that the strain both stimulates the growth of melon seedlings and significantly inhibits the growth of the *Fusarium oxysporum* melon specialized type. *T. viride* kf57 strain is considered safe and does not require strain safety testing because it is derived from natural soil.

### 2.2 Soil characteristics

The soil used in the seedling-phase experiment came from a greenhouse where melons were continuously planted for 3 years. The soil is suitable for usage after it has been filtered using a 1 mm sieve. The basic agrochemical characteristics of the soil used for seedling testing were are shown in Table 1. Melons were grown continuously for 3 years in greenhouses at the fruit-phase experiment.

TABLE 1 Fundamental agrochemical characteristics of soils that have been grown for 3 years with melon.

Index	Value
Organic matter	33.81 g/kg
Alkali-hydrolyzed nitrogen	163.53 mg/kg
Available phosphorus	25.62 mg/kg
Available potassium	236.14 mg/kg
pH	7.5

### 2.3 Preparation of *Trichoderma viride* conidia powder

*Trichoderma viride* was cultured on PDA culture material for 3 days at 28°C in the dark as an activation culture. To make the *Trichoderma* spore solution, five samples from the margins of each colony, each measuring 5 mm in diameter, were collected. After that, these samples were put into PDA culture media and kept at 28°C in the dark for 7 days. Then, distilled water was used to cleanse the spores before they were extracted. After diluting it with a sterile water gradient, it is coated over the *Trichoderma* selective medium and inverted for a couple of days at 25–28°C. By counting the colonies, determine the number of *Trichoderma* conidia. For a full night, clean, room-temperature water was used to soak barley grains, sterilizing them. One kilogram of fresh-keeping bags were then filled with the remaining mixture after the water was removed. After chilling, suspensions of *Trichoderma viride* were added, and they were cultivated for 2–3 weeks at 25°C. When the spore has gotten too big, rinse with sterile water and filter the grain away. After adding 10% talcum powder, the filtrate was crushed, filtered, and dried to produce *Trichoderma viride* conidium powder. With  $1.1 \times 10^{10}$  CFU/g of *Trichoderma viride* conidiomycetes, the administered dose was calculated in compliance with the test requirements.

### 2.4 Experimental design and treatments

At seedling test, the melon cultivar Longtian No. 1 from Xiangnong Seed Corporation Ltd., Haerbin, Heilongjiang, China, was used in this investigation. The Heilongjiang Bayi Agricultural University's teaching facility in China provided the plastic greenhouse where this experiment was conducted. The soil from the greenhouse where melons were continuously planted for 3 years for seedlings was then placed in plastic seedling culture plates (30.5 cm × 20.5 cm × 8.6 cm), each of which contains 5.25 kg of soil along with varying concentrations of *Trichoderma viride* conidia agents. Following germination treatment, 140 melon seeds were added to each plate, and 100 seedlings of a similar size were saved. After sowing, melons were given 1,000 mL of water every 2 days to maintain a normal growth stage.

Each of the five treatments in this study contained 100 seedlings spread across five dishes. Four times the experiment was run, with a different set of seedlings being chosen at random each time.

The following were the treatment groups:

(1)  $1.0 \times 10^4$  cfu/g *Trichoderma viride* conidia agents (T1); (2)  $8.0 \times 10^4$  cfu/g *Trichoderma viride* conidia agents (T2); (3)  $6.4 \times 10^5$  cfu/g *Trichoderma viride* conidia agents (T3); (4)  $5.12 \times 10^6$  cfu/g *Trichoderma viride* conidia agents (T4); (5) No *Trichoderma viride* agents (CK) were applied.

The root-shoot ratio and strong seedling index, as well as seedling morphological indices and material accumulation indices for melon seedlings, were calculated for 40 plants from each treatment at 30 days after sowing (10 plants per replication) high furrow cultivation.

At fruiting stage test, the soil at the plastic greenhouse was fertilized with 225 kg of diammonium phosphate, 300 kg of potassium sulfate, and 375 kg of organic fertilizer per acre. The ground was completely prepared for beds. Choose healthy seedlings that have steady growth and place them in a plastic greenhouse where melons were grown continuously for 3 years on April 25, 2022, when the melon seedlings have reached the size of three leaves and

one heart. Bedding cultivation is used in plastic greenhouses with a randomized block arrangement. The experimental design is the same as in 1.3.1, with four replicates. Place a blank space between each bed. The bed measures 6 meters in length, 1.0 meters in width, 1.2 meters in width, 1:100 in slope, and 10 cm in depth. The cultivation of melon in the top beds uses double row planting, with 15 plants placed in each bed with a plant spacing of 40 cm and a row spacing of 50 cm.

After a delayed seedling, the melon was selected on the fourth leaf, grown on two vines, and watered every 3 days. When the seventh leaf was extended, the plants started to hang. Double vines are used to prune the melon plant, and after about 2–3 knots, the fruit vines begin to grow continually. Select 3–4 uniform and sturdy fruits from each melon plant when the fruit's longitudinal diameter reaches 2–3 cm, discard the other fruits, and pluck the heart from the vine's 20–25 leaves.

Female flowers from the same node and blossoming time were used to label 75 plants on the day of pollination. Sampling started on the tenth day following pollination, and it was done five times in total, once every 5 days. In the adult plant stage, 4–5 leaves on the fruit vine were used as the aim source from 9:00 to 10:00 a.m. to determine the physiological and biochemical indices. At 30 days following pollination, 16 fruits (4 fruits per replication) were randomly chosen from each treatment, and the quality indices were assessed using mixed fruit samples. In addition, the plot yield and hectare yield were determined while 16 randomly chosen fruits (4 fruits per replication) from each treatment were measured for fruit transverse diameter, longitudinal diameter, and single melon weight.

## 2.5 Measured parameters

### 2.5.1 Determination of morphological indicators

The distance between the stem base and its growth point on each seedling was measured with a ruler to calculate the plant height (Shoam et al., 2022). By measuring the diameter of the stem 1 cm below the cotyledon with a vernier caliper, the diameter of the stem was determined (Shoam et al., 2022).

By using the weighing method, the leaf area was calculated (Wang et al., 2021). A leaf with a particular area (A1) was first gathered and layered with other leaves. Next, round sheets were created using a 1-cm puncher pin. The weights of the round sheets and the remaining leaves were then measured, and from there, the weight of the leaf with a particular area (W1) and the weights of the other leaves (W2) were computed. The formula  $A = [A1(W1 + W2)]/W1$  was used to calculate the leaf total area (A) based on these measurements.

### 2.5.2 Determination of material accumulation indicators

The plants were periodically cleaned with clear water before being dried on absorbent paper. The fresh weight of the plants was computed based on their above- and below-ground parts. After that, the fresh samples were baked to a consistent weight at 70°C after being dried at 105°C for 15 min. The above- and below-ground components' dry weights were calculated using an electronic scale with a resolution of 1/1,000 (Jang et al., 2021).

Using Gou et al. (2022) method, the root-shoot ratio was calculated using the following formula:

Root-shoot ratio = fresh weight of underground part/fresh weight of above-ground part.

The strong seedling index was calculated using the following formula in accordance with Liu X. J. et al. (2022) methodology:

Strong seedling index = (Stem diameter/Plant height + underground dry weight/above ground dry weight) × dry weight of whole plant.

### 2.5.3 Determination of physiological and biochemical indicators

The ethanol technique was used to determine the content of chlorophyll (Ahmad et al., 2022). For nitrate nitrogen content, the phenolic disulfonic acid technique was utilized (Zheng et al., 2018). The anthrone colorimetric method was used to estimate the content of sucrose (Song et al., 2011). The 3, 5-dinitrosalicylic acid technique was used to determine the content of reducing sugar (Xu et al., 2022). By using sulfosalicylic acid in an acid ninhydrin extraction, the content of free proline (Pro) was discovered (Chong and Jun, 2005). *In vivo* spectrophotometry was used to evaluate nitrate reductase (NR) activity (Rong et al., 2018). The guaiacol technique was used to assess the peroxidase (POD) activity (Zhang Q. et al., 2019). The nitrogen blue tetrazole photochemical reduction technique was used to examine the activity of superoxide dismutase (SOD) (Hussain et al., 2017).

### 2.5.4 Determination of fruit quality indicators

The content of soluble solids was calculated using an Abel refractometer. The anthrone colorimetric method was used to quantify the content of soluble sugar (Song et al., 2011). The Coomassie Brilliant Blue G-250 staining procedure was used to quantify the content of soluble protein (Zheng et al., 2018). UV spectrophotometry was used to assess the vitamin C content (Kampfenkel et al., 1995). Using the 0.1 mol/L sodium hydroxide standard solution technique, the total acid content was titrated.

### 2.5.5 Determination of yield indicators

Vernier calipers were used to measure the transverse and longitudinal diameters of melon fruit. A single melon fruit was weighed using an analytical balance (TB-4002, BeiXiaogan Matguang Medical Electronics Co., Ltd., Xiaogan, Hubei, China), with an accuracy of 0.01 g.

## 2.6 Statistical analysis

The DPS 7.05 program was used for data statistics and variance analysis. Duncan's distinct challenging range technique was employed to assess multiple comparisons across treatments ( $p < 0.05$ ). Origin 2019 software was used for mapping.

## 3 Results

### 3.1 Effect of *Trichoderma viride* agents on the morphology of melon grown on soils that were continuously cropped to melon seedlings

The root-shoot ratio and strong seedling index were calculated on day 30 after sowing using the morphological indices of plant height, stem diameter, leaf area, and material accumulation indices of fresh

weight and dry weight in the aboveground and underground parts of melon grown on soils continuously cropped to melon seedlings treated with various *Trichoderma viride* agents. The results are shown in Table 2.

Melon seedlings treated with  $1.0 \times 10^4$ ,  $8.0 \times 10^4$ ,  $6.4 \times 10^5$  and  $5.12 \times 10^6$  cfu/g *Trichoderma viride* agents had considerably greater morphogenetic indices and material accumulation indices than those treated with CK (no *Trichoderma viride* agents were used). The best effects on plant height, stem diameter, leaf area, fresh weight, dry weight, root-shoot ratio, and strong seedling index were seen with T3 ( $6.4 \times 10^5$  cfu/g *Trichoderma viride* agents). It grew by 90.39, 46.30, 37.55, 81.35, 100.62, 51.47, and 240.00% when compared to CK, respectively. These findings suggested that *Trichoderma viride* at the right concentration could encourage melon grown on soils continuously cropped to melon seedling morphology.

## 3.2 Effect of *Trichoderma viride* agents on physiological and biochemical indices of melon grown on soils continuously cropped to melon during its fruiting stage

### 3.2.1 The contents of chlorophyll and nitrate nitrogen

The chlorophyll and nitrate nitrogen content of melon leaves treated with *T. viride* agents during the fruiting stage exhibited a trend of first increasing and then decreasing with the passage of pollination time, with peaks occurring 25 days after pollination, as seen in Figures 1A,B. At 10, 15, 20, 25, and 30 days after pollination, the chlorophyll and nitrate nitrogen content of melon leaves showed an increasing trend followed by a decreasing trend with the increase of *T. viride* agents treatment concentration. T3 melon leaves had the highest levels of both chlorophyll and nitrate nitrogen. Melons treated with T3 had the maximum chlorophyll content at 10, 15, 20, 25, and 30 days following pollination, as seen in Figure 1A. The chlorophyll content of melon leaves rose by 39.30, 38.43, 41.94, 35.49, and 34.68%, respectively, when treated with T3 as opposed to CK. According to Figure 1B, the T3 treatment had the highest nitrate nitrogen content in melon leaves at 10, 15, 20, 25, and 30 days following melon pollination, which was noticeably higher than that of the other treatments. The nitrate nitrogen concentration of melon leaves rose by 34.97, 46.91, 75.86, 117.03, and 115.05% during T3 treatment as opposed to CK.

### 3.2.2 The contents of sucrose and reducing sugar

Melons treated with *T. viride* agents during the fruiting stage exhibited a trend of initially increasing and then declining with the passage of pollination time, with peaks occurring 25 days following pollination, as illustrated in Figures 2A,B. The sucrose and reducing sugar content in melon leaves indicated a trend of initially rising and then falling with the increase in *T. viride* agents treatment concentration at 10, 15, 20, 25, and 30 days after melon pollination. In terms of sucrose and decreasing sugar content, T3 melon leaves were the greatest. Figures 2A illustrates that at 10, 15, 20, 25, and 30 days following melon pollination, T3 treatment produced the highest sucrose content in melon leaves, increasing the sucrose content by 35.73, 38.23, 34.71, 31.27, and 33.47%, respectively, in comparison to CK. Figures 2B displays the highest content of reducing sugars in melon leaves at 10, 15, 20, 25, and 30 days following melon pollination, increasing the reducing sugar content in melon leaves by 33.38, 28.49, 25.42, 42.45, and 27.33%, respectively.

### 3.2.3 Free proline content and nitrate reductase activity

The amount of free proline in melon leaves treated with *T. viride* agents during the fruiting stage exhibited a trend of steadily rising levels as pollination time passed, as illustrated in Figures 3A,B. During the fruiting phase treated with *T. viride* agents, the NR activity of melon leaves exhibited a tendency of first rising and then falling with the passage of pollination time, peaking 25 days after pollination. The amount of free proline and NR activity in melon leaves at 10, 15, 20, 25, and 30 days after pollination exhibited a trend of initially rising and then falling as the concentration of *T. viride* agents treated increased. The highest levels of free proline and nitrate reductase activity were seen in T3 melon leaves. The T3 treatment had the maximum amount of free proline in the melon leaves at 10, 15, 20, 25, and 30 days following pollination, as seen in Figure 3A. T3 treatment raised the amount of free proline in melon leaves by 51.95, 26.46, 26.63, 50.98, and 26.44%, respectively, in comparison to CK. The NR activity of T3 melon leaves was the highest and considerably higher than that of other treatments at 10, 15, 20, 25, and 30 days after melon pollination, as illustrated in Figure 3B. Melon leaves' NR activity rose by 56.21, 53.91, 48.48, 39.92, and 37.84% with T3 treatment, respectively, in comparison to CK.

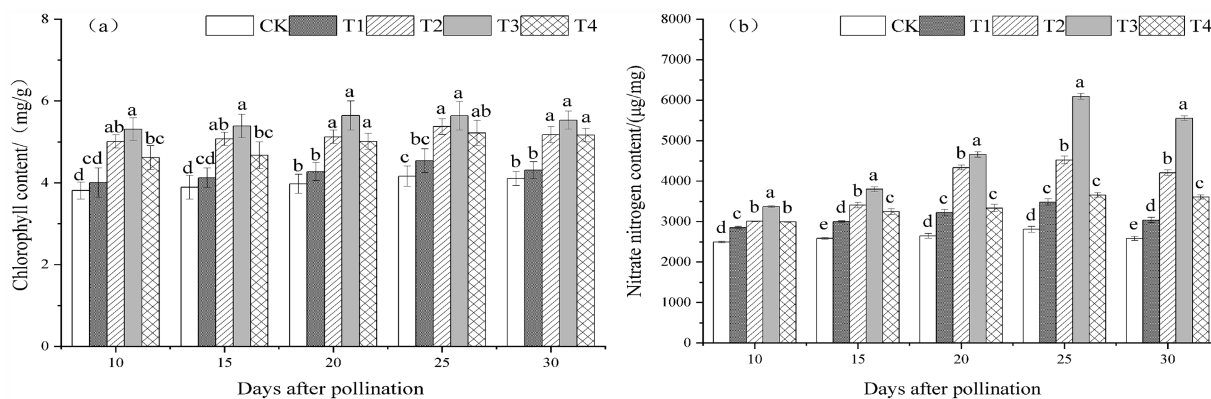
### 3.2.4 The activities of peroxidase and superoxide dismutase

The POD and SOD activities of melon leaves treated with *T. viride* agents during the fruiting stage exhibited a trend of first rising and then

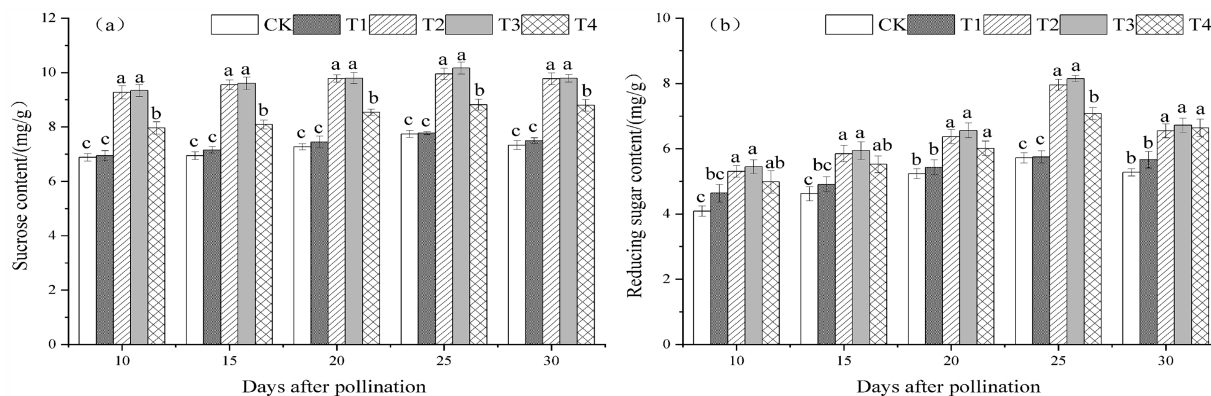
TABLE 2 Morphological parameters of melon seedlings under various *T. viride* agents application rates.

Treatment	Plant height (cm)	Stem diameter (cm)	Leaf area (cm <sup>2</sup> )	Fresh weight of whole plant (g)	Dry weight of whole plant (g)	Root shoot ratio	Strong seedling index
CK	9.625 ± 0.362d	0.311 ± 0.012c	20.126 ± 1.426d	2.204 ± 0.084e	0.162 ± 0.006d	0.068 ± 0.003d	0.015 ± 0.003e
T1	13.247 ± 0.568c	0.357 ± 0.008b	21.584 ± 1.595c	2.640 ± 0.071d	0.200 ± 0.008c	0.078 ± 0.004c	0.020 ± 0.002d
T2	16.237 ± 0.714b	0.392 ± 0.018a	25.314 ± 1.633b	3.629 ± 0.062b	0.287 ± 0.005b	0.096 ± 0.005b	0.039 ± 0.007b
T3	18.325 ± 0.832a	0.455 ± 0.027a	27.683 ± 1.429a	3.997 ± 0.057a	0.325 ± 0.004a	0.103 ± 0.006a	0.051 ± 0.006a
T4	14.635 ± 0.638c	0.378 ± 0.016b	22.519 ± 1.574c	3.032 ± 0.041c	0.238 ± 0.004c	0.093 ± 0.008b	0.029 ± 0.003c

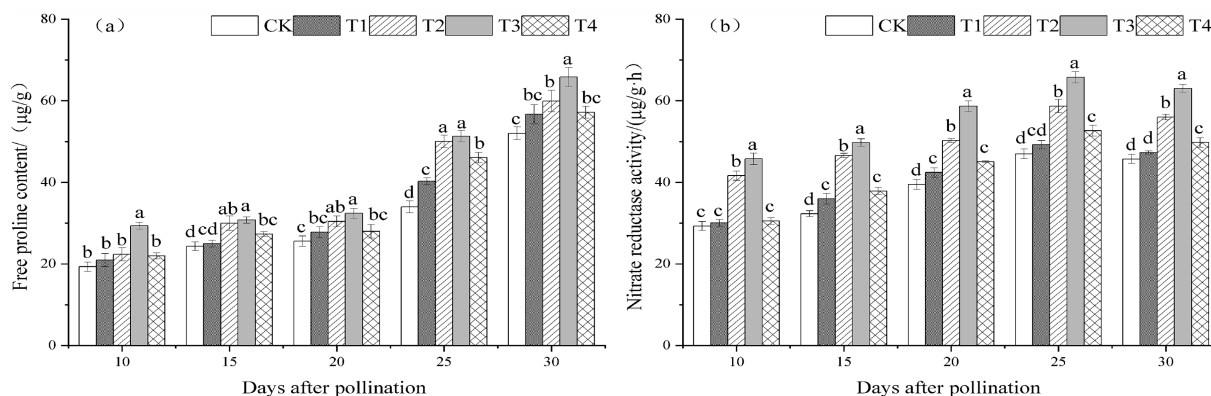
In the table, the values show the mean ± SD, and different lowercase letters in the same column indicate significant differences at the 5% ( $p < 0.05$ ) level. CK, No *T. viride* agents were applied; T1,  $1.0 \times 10^4$  cfu/g *T. viride* conidia agents; T2,  $8.0 \times 10^4$  cfu/g *T. viride* conidia agents; T3,  $6.4 \times 10^5$  cfu/g *T. viride* conidia agents; T4,  $5.12 \times 10^6$  cfu/g *T. viride* conidia agents.



**FIGURE 1** Chlorophyll content (A) and nitrate nitrogen content (B) under different ratios of *T. viride* agents in leaves during melon fruiting stage. Different lowercase letters indicate significant differences among the 5 treatments from 10 to 30 days after pollination at the 0.05 level ( $p < 0.05$ ). CK, No *T. viride* agents were applied; T1,  $1.0 \times 10^4$  cfu/g *T. viride* conidia agents; T2,  $8.0 \times 10^4$  cfu/g *T. viride* conidia agents; T3,  $6.4 \times 10^5$  cfu/g *T. viride* conidia agents; T4,  $5.12 \times 10^6$  cfu/g *T. viride* conidia agents.



**FIGURE 2** Sucrose content (A) and reducing sugar content (B) under different ratios of *T. viride* agents in leaves during melon fruiting stage. Different lowercase letters indicate significant differences among the 5 treatments from 10 to 30 days after pollination at the 0.05 level ( $p < 0.05$ ). CK, No *T. viride* agents were applied; T1,  $1.0 \times 10^4$  cfu/g *T. viride* conidia agents; T2,  $8.0 \times 10^4$  cfu/g *T. viride* conidia agents; T3,  $6.4 \times 10^5$  cfu/g *T. viride* conidia agents; T4,  $5.12 \times 10^6$  cfu/g *T. viride* conidia agents.



**FIGURE 3** Free proline content (A) and nitrate reductase activity (B) under different ratios of *T. viride* agents in leaves during melon fruiting stage. Different lowercase letters indicate significant differences among the 5 treatments from 10 to 30 days after pollination at the 0.05 level ( $p < 0.05$ ). CK, No *T. viride* agents were applied; T1,  $1.0 \times 10^4$  cfu/g *T. viride* conidia agents; T2,  $8.0 \times 10^4$  cfu/g *T. viride* conidia agents; T3,  $6.4 \times 10^5$  cfu/g *T. viride* conidia agents; T4,  $5.12 \times 10^6$  cfu/g *T. viride* conidia agents.

falling with the passage of pollination time, with peaks occurring 25 days after pollination, as seen in Figures 4A,B. The POD and SOD activities of melon leaves exhibited a tendency of initially rising and then falling with the increase in *T. viride* agent treatment concentration at 10, 15, 20, 25, and 30 days following melon pollination. T3 melon leaves had the highest POD and SOD activity. Figure 4A illustrates that the POD activity of T3 melon leaves was the highest and substantially higher than that of other treatments at 10, 15, 20, 25, and 30 days following melon pollination. Melon leaves' POD activity rose by 54.22, 48.29, 35.05, 73.97, and 61.20% with T3 treatment, respectively, in comparison to CK. Figure 4B illustrates that the SOD activity of T3 melon leaves peaked 10, 15, 20, 25, and 30 days after melon pollination. Melon leaves' SOD activity rose by 78.20, 50.01, 28.33, 18.62, and 22.60% with T3 treatment, respectively, in comparison to CK.

### 3.3 The effect of *Trichoderma viride* agents on yield and quality of melon grown on soils that were continuously cropped to melon

#### 3.3.1 The effect of *Trichoderma viride* agents on yield traits of melon

After 30 days of pollination, melon fruit weight, transverse diameter, and longitudinal diameter were measured under various *T. viride* agents treatments, and melon yield was computed, as shown in Table 3. As the concentration of *T. viride* agent treatment increased, the transverse diameter, longitudinal diameter, single fruit weight, and yield of melon fruits treated with the agent exhibited a trend of initially increasing and then dropping. With transverse diameter of 8.621 cm, longitudinal diameter of 13.106 cm, single fruit weight of 521.726 g, and yield of 26321.254 kg/hm<sup>2</sup>, T3 was the largest. In comparison to CK, T3 rose by 27.87, 39.81, 62.23, and 93.99%, respectively.

#### 3.3.2 The effect of *Trichoderma viride* agents on quality of melon

A variety of melon quality indices were measured 30 days after pollination using various *T. viride* agents treatments. The results are

displayed in Table 4. As the concentration of *T. viride* agent treatment increased, the soluble solids content, vitamin C content, soluble protein content, soluble sugar content, and sugar acid ratio of melon fruit treated with *T. viride* agents showed an increasing trend, followed by a decreasing trend. The T3 fruit exhibited the highest levels of soluble solids, vitamin C, soluble protein, soluble sugar, and sugar acid ratio, with respective contents of 12.064%, 266.381 mg/100 g, 1294.746 mg/kg, 64.095 mg/g, and 15.493. Comparing T3 to CK, the increases were 50.16, 58.07, 85.56, 50.03, and 90.82%, respectively. Compared to CK, T3 fruit had the lowest total acid content (4.137 g/kg), which was 27.19% lower.

## 4 Discussion

Melon's root vitality is impeded and its weight and growth of roots and leaves are greatly reduced as a result of long-term continuous cropping (Gava and Pinto, 2016). This leads to a significant degradation of the soil's physical and chemical properties, reduced organic matter content, and an imbalanced soil nutrient (Xu et al., 2024). Simultaneously, the build-up of autotoxic chemicals in soil alters the soil microenvironment, impacts physiological functions as crop photosynthesis and enzyme activity, and prevents crop growth (Du et al., 2024). *Trichoderma*, a popular biological control agent, can successfully reduce ongoing cropping barriers in the production of melon (Ruangwong et al., 2021).

*Trichoderma* can spread quickly in soil, persist for a long period on the surface of plant roots, and release a number of chemicals (Raj and Sharma, 2009) that can aid in the growth of plants. This study's findings demonstrated that *T. viride* agents treatment might stimulate the morphogenesis of melon seedlings, with T3 (6.4 × 10<sup>5</sup> cfu/g) having the most favorable results. *Trichoderma viride* Tv 911 was shown by Zaw and Matsumoto (2020) to promote the growth of Japanese mustard, tomato, and radish. As a result, the plant height of mustard and tomato increased by 16.22 and 50.26%, respectively, and the fresh branch and root weight of radish increased by 23.83 and 58.86%, respectively. This is so because *Trichoderma* regulates the physiological and biochemical metabolism processes of plants (Vinale

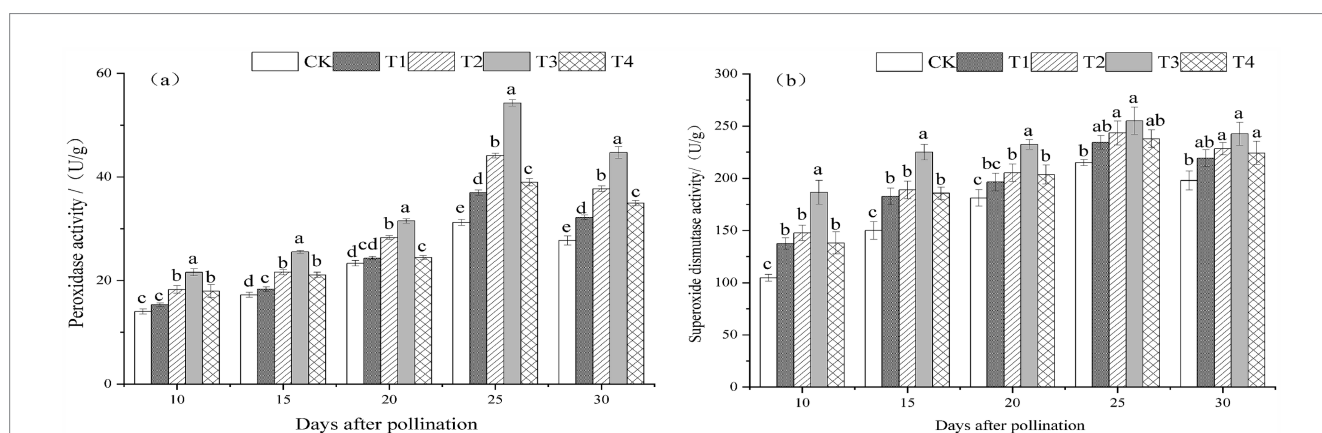


FIGURE 4 Peroxidase activity (A) and superoxide dismutase activity (B) under different ratios of *T. viride* agents in leaves during melon fruiting stage. Different lowercase letters indicate significant differences among the 5 treatments from 10 to 30 days after pollination at the 0.05 level ( $p < 0.05$ ). CK, No *T. viride* agents were applied; T1,  $1.0 \times 10^4$  cfu/g *T. viride* conidia agents; T2,  $8.0 \times 10^4$  cfu/g *T. viride* conidia agents; T3,  $6.4 \times 10^5$  cfu/g *T. viride* conidia agents; T4,  $5.12 \times 10^6$  cfu/g *T. viride* conidia agents.

TABLE 3 Yield traits of melon under various *T. viride* agents application rates.

Treatment	Fruit transverse diameter (cm)	Fruit vertical diameter (cm)	Average single fruit weight (g)	Yield (kg/ hm <sup>2</sup> )
CK	6.742 ± 0.084d	9.374 ± 0.342d	321.587 ± 3.587c	13568.274 ± 632.564c
T1	7.308 ± 0.065c	9.915 ± 0.101c	381.362 ± 1.362b	17638.262 ± 863.954b
T2	8.463 ± 0.062a	11.007 ± 0.131b	516.328 ± 2.358a	23957.674 ± 903.625a
T3	8.621 ± 0.058a	13.106 ± 0.801a	521.726 ± 3.698a	26321.254 ± 869.362a
T4	7.751 ± 0.085b	10.326 ± 0.126bc	394.304 ± 2.684b	19586.681 ± 963.625b

In the table, the values show the mean ± SD, and different lowercase letters in the same column indicate significant differences at the 5% ( $p < 0.05$ ) level. CK, No *T. viride* agents were applied; T1,  $1.0 \times 10^4$  cfu/g *T. viride* conidia agents; T2,  $8.0 \times 10^4$  cfu/g *T. viride* conidia agents; T3,  $6.4 \times 10^5$  cfu/g *T. viride* conidia agents; T4,  $5.12 \times 10^6$  cfu/g *T. viride* conidia agents.

TABLE 4 Quality traits of melon under various *T. viride* agents application rates.

Treatment	Soluble solids content (%)	Vitamin C content/ (mg/100 g)	Soluble protein content (mg/kg)	Total acid content (g/kg)	Soluble sugar content(mg/g)	Sugar-acid ratio
CK	8.034 ± 0.089d	168.526 ± 6.097d	697.749 ± 10.541c	5.262 ± 0.011a	42.722 ± 0.962c	8.119 ± 0.108c
T1	9.343 ± 0.053c	195.082 ± 8.370c	885.138 ± 12.969b	4.677 ± 0.009c	51.728 ± 1.325b	11.060 ± 0.163b
T2	11.036 ± 0.058b	249.289 ± 5.324a	1250.269 ± 77.845a	4.223 ± 0.019d	61.058 ± 1.385a	14.458 ± 0.174a
T3	12.064 ± 0.442a	266.381 ± 7.068a	1294.746 ± 33.544a	4.137 ± 0.014d	64.095 ± 1.652a	15.493 ± 0.214a
T4	10.949 ± 0.093b	223.390 ± 5.725b	926.767 ± 45.695b	4.935 ± 0.017b	54.745 ± 1.847b	11.093 ± 0.141b

In the table, the values show the mean ± SD, and different lowercase letters in the same column indicate significant differences at the 5% ( $p < 0.05$ ) level. CK, No *T. viride* agents were applied; T1,  $1.0 \times 10^4$  cfu/g *T. viride* conidia agents; T2,  $8.0 \times 10^4$  cfu/g *T. viride* conidia agents; T3,  $6.4 \times 10^5$  cfu/g *T. viride* conidia agents; T4,  $5.12 \times 10^6$  cfu/g *T. viride* conidia agents.

et al., 2008), which in turn promotes plant growth (Elkelish et al., 2020).

Plants' physiological metabolism can be enhanced by *Trichoderma*, in addition to its growth-promoting effects (Harman et al., 2004). The content of physiological chemicals such as MDA and Pro is impacted by the secondary metabolites, such as CAT, POD, APX, SOD, and others, that *Trichoderma* secretes during plant-microbe contact. Plants may become resistant to these chemicals on a systemic level (Gajera et al., 2016). According to the study's findings, application of the *T. viride* agents improved the physiological properties of melons, with application of T3 having the greatest impact. According to Yu et al. (2021), *T. asperellum* spore solution raised the amount of soluble protein and soluble sugar in leaves, enhanced the activity of nitrate reductase and catalase, and boosted the plant height and stem thickness of tomato seedlings. According to Ahmad et al. (2022) research, *T. harzianum* Th23 treatment could boost tomato growth, increase the total chlorophyll content of leaves, and boost the activities of protective enzymes like PPO, CAT, and SOD (Abdelkhalek et al., 2022). According to Cao et al. (2022) the growth of continuous cropping cucumber was enhanced by the treatment of both biochar and *Trichoderma* in combination. There was a notable increase in root activity as well as the activities of POD, CAT, and SOD in leaves, along with a decrease in the amount of MDA. This is due to the fact that *Trichoderma* is a biological control pathogen that also stimulates plant development. In addition, its secondary metabolites have the ability to create hormone analogs and plant growth regulators, both of which stimulate plant growth (Vinale et al., 2012). This is in line with research conducted by Martínez-Medina et al. (2014) on the impact of four distinct strains of *Trichoderma* on the biological control activity of melon wilt disease and the promotion of plant growth. The outcomes demonstrated a substantial association between

*Trichoderma* induction of auxin and stimulation of plant development, as well as an increase in auxin content that stimulated plant growth and a decrease in cytokinin and abscisic acid content.

The findings of this study demonstrated that *T. viride* agents treatment enhanced the physiological properties of melons and encouraged the development of melon yield and quality, with T3 treatment having the most favorable results. The results of a study by Liu L. et al. (2022) on the effects of *T. harzianum* biofilter on the growth, yield, and quality of bupleurum as well as the microbial reaction revealed that *T. harzianum* biofilter encouraged bupleurum growth and increased its yield and quality. Bupleurum had an increase in plant height, stem diameter, root diameter, and total plant weight compared to the control, respectively, of 20.52, 21.82, 38.36, and 126.70%. Saikosaponins A, C, and D levels in Bupleurum were also significantly higher thanks to *T. harzianum* bio-fertilizer, increasing by 8.06, 47.73, and 9.23%, respectively, in comparison to the control. According to Zhang et al. (2023), *T. viride* T23 enhanced the microbial community's biomass and diversity, enhanced the physical and chemical characteristics of the soil, boosted the synthesis of soil-active substances, removed a barrier to melon continuous cropping, and encouraged the formation of melon yield. All of these support Fernando et al. (2018) finding that the two isolates of *Trichoderma* can boost melon production by promoting melon seed germination and seedling growth.

*Trichoderma* is a common antagonistic microorganism found in nature that can help plants absorb nutrients, enhance the soil environment, speed up the breakdown of organic matter in the soil, and boost crop quality and yield (Rudresh et al., 2005; Wonglom et al., 2020; Harman, 2006). Similar to the findings of Lu et al. (2022), the results of this study showed that *T. viride* applied in varying amounts can promote the formation of melon yield and quality, with T3 ( $6.4 \times 10^5$  cfu/g) treatment having the best promoting effect. The



yield and quality also decreased as the application amount increased. According to Lu et al., applying the *Trichoderma* microbial agents might boost the output of *Lycium barbarum*, and the A4 treatment (74.25 kg·hm<sup>-2</sup>) produced the highest results. *Trichoderma* microbial agents were used to increase the amounts of soluble solids, *lycium barbarum* polysaccharide, betaine, total flavonoids, and carotenoids in *Lycium barbarum* fruits; the A3 (59.40 kg·hm<sup>-2</sup>) treatment had the greatest impact. In contrast to traditional substrate treatment, Wang et al. (2025) discovered that *Trichoderma* mixes at varying concentrations can considerably accelerate the growth of watermelon seedlings. The botanical characteristics and material accumulation of watermelon seedlings exhibit a trend of initially growing and then decreasing as the concentration of *Trichoderma* mixes rise. In comparison to the other treatments, the T3 (10<sup>6</sup> CFU/g) treatment resulted in higher plant height, stem diameter, fresh mass above ground, dry mass above ground, root length, fresh mass below ground, dry mass below ground, root surface area, number of tips, and root shoot ratio. The results of this study showed that the best concentration for melon growth was 6.4 × 10<sup>5</sup> CFU/g, which may be attributed to the necessity to maintain a specific degree of diversity and balance in the soil microbial community. Even helpful bacteria, excessive development can squeeze the living space of other microorganisms, resulting to a decline in soil ecosystem variety, which is not conducive to soil health and crop growth (Masquelier et al., 2022). Therefore, it is essential to regulate the volume of application while using various *Trichoderma* microbial agents. When *Trichoderma* microbial agents were applied more frequently, plant production and quality rose as well. However, it would seem that this effect is a threshold phenomenon. In other words, too much *Trichoderma* microbial agents were used, which would have reduced plant quality and productivity. Excessive application levels will also result in higher expenses; if promoted widely, *Trichoderma* microbial agents will be wasted (Li et al., 2018; Lian et al., 2021).

Numerous researchers have found that *Trichoderma* has a strong capacity to colonize soil (Zhao et al., 2023), enhance soil physicochemical conditions (Zhu et al., 2022), boost soil fertility (Fu et al., 2019), encourage the formation and upkeep of beneficial microbial communities in soil (Zhang et al., 2018), and increase crop development and yield. The effects of *T. viride* kf57 conidia agents on the soil environment of melon cultivated on soils that have been constantly cropped to melon will be further investigated in future studies.

## 5 Conclusion

Overall, our research showed that different *T. viride* kf57 agent application rates improve the root shoot ratio and seedling strength index of melon seedlings, improve the morphological development at the seedling stage and the physiological and biochemical characteristics at the fruiting stage, and increase the yield and quality of melon grown on soils that are continuously cropped to melon. The most effective of these is the application of 6.4 × 10<sup>5</sup> cfu/g *T. viride* kf57 agent. The findings of the study offer fresh concepts for overcoming the challenges associated with melons' continuous production and encouraging the melon industry's sustainable growth. Additionally, they offer physiological mechanisms for the quick study and development of

*Trichoderma* agents as well as a foundation for research to guarantee the high-quality and high-yield production of melons grown on soils that are consistently cropped with *Trichoderma* agents.

## Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

## Author contributions

JLiu: Conceptualization, Data curation, Investigation, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. HL: Conceptualization, Methodology, Project administration, Supervision, Writing – review & editing. JD: Data curation, Investigation, Writing – review & editing. JLi: Data curation, Investigation, Resources, Writing – original draft. GZ: Investigation, Resources, Writing – review & editing. JW: Conceptualization, Data curation, Investigation, Writing – original draft. GM: Conceptualization, Methodology, Project administration, Supervision, Writing – review & editing. ML: Funding acquisition, Project administration, Supervision, Writing – review & editing.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fsufs.2025.1513324/full#supplementary-material>

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