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ORIGINAL PAPER

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EFFECTS OF ORGANIC CULTIVATION PATTERN ON TOMATO PRODUCTION: PLANT GROWTH CHARACTERISTICS, QUALITY, DISEASE RESISTANCE, AND SOIL PHYSICAL AND CHEMICAL PROPERTIES

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ABSTRACT

Tomato (*Solanum lycopersicum* L.) is one of the world's most important cultivated vegetable. In the traditional cultivation methods, the excessive use of pesticides and fertilizers leads to an imbalance of nutrient elements in the soil, an increase in pests and diseases and a decrease in vegetable quality and yield. In the face of increasingly serious environmental and food problems, organic agriculture is considered to be an effective solution. In this experiment, the effects of organic cultivation patterns on the growth, quality, disease resistance in tomatoes, and the physical and chemical properties of soil were studied by different treatments. The results showed that the application of effective microorganisms (EM) bio-organic fertilizer in the cultivation process can significantly improve the yield, quality, and antioxidant enzyme activity of tomato. The use of straw mulching was found to significantly increase the growth, chlorophyll content, transpiration rate, and soluble sugar content of tomatoes. The application of EM bio-organic fertilizer or straw mulching significantly increased the activity of antioxidant enzyme and the expression of *LeCHI* gene in tomato leaves and enhanced tomato resistance to diseases. Organic production practices were found to significantly improve the soil.

Key words: organic production, tomato, growth characteristics, disease resistance, soil physical and chemical properties

INTRODUCTION

Tomato is one of most economically important vegetable crop in the world [Xiong et al. 2017]. Tomato fruit is nutrient-rich, and contains vitamin C, calcium, iron, phosphorus, and other minerals involved in cardiovascular protection. Tomato lycopene and carotene are powerful antioxidants that can effectively reduce the incidence of various cancers [Martínezvalverde et al. 2002, Pinela et al. 2012]. With the expansion of the tomato cultivation area and the continuous increase in consumption level, consumers place more demands on the quality, nutritional content and taste of tomatoes [Beckles 2012]. In tomato production, an increase in diseases and insects and poor soil often result in reduced production. In order to solve these problems, chemical fertilizers and pesticides are widely used in the production of tomatoes. However, the long-term

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use of chemical fertilizers and pesticides and irrational production and management measures have led to the breakage of the micro-ecological environment of the soil, which causes physiological diseases of vegetables, leading to the decline in production and quality, and to economic losses [Tomkiel et al. 2017]. Therefore, studying the rational and efficient application of organic production practices of tomato is an important subject that modern agriculture needs to address.

In accordance with the standards of organic agriculture, organic practices are not allowed to use synthetic fertilizers, pesticides, hormone and genetic engineering, ion radiation. In the production process, only material from animal origin, plant origin, or mined, are allowed to maintain soil fertility and reduce the occurrence of pests and diseases. Thus, methods such as bio-organic fertilizer and straw mulching have a wide range of applications in the production of organic agriculture.

Effective microorganisms (EM) bio-organic fertilizer is both a microbial fertilizer and an organic fertilizer at the same time. It is composed of organic waste, such as plant residue, cake, and animal feces; and multi-compound functional microbial agents by fermentation, deodorization, and complete maturity. Higa, at the University of Ryukyu, Japan, discovered and isolated a large number of beneficial microorganisms from the soil, developed new compound microbial agent EM products that may be applied in organic agriculture. EM bio-organic fertilizer contains more than 80 kinds of microorganisms, such as photosynthetic bacteria, lactic acid bacteria, yeasts, actinomycetes and filamentous bacteria. [Higa and Wididana 1991, Teruo 1991]. Compared with chemical fertilizer, EM bio-organic fertilizer not only contains the nutrients that are necessary to the plants, organic compounds, and growth active substances, but also has a wide range of raw materials, abundant in quantity [Tomkiel et al. 2017, Moon et al. 2018]. Denison et al. [2004] reported that tomato production in an organic farming system was increasing year by year with use of EM fertilizer. Hallmann et al. [2012] determined the quality of five organic and conventional cultivated tomatoes and found that the titratable acid of the five tomato cultivars was not significantly different. They also reported that vitamin C and soluble sugar content of organic tomato were higher than those of conventional tomato. Jia et al. [2010] and Ling et al. [2011] reported that EM bio-organic fertilizers contain a large number of beneficial microorganisms. The also reported that the fertilizer improved the crop's resistance to diseases, reduced the number of soil pests, controlled soil-born diseases of plants. Tian et al. [2001a] applied EM bio-organic fertilizers and chemical fertilizers to tomatoes respectively, and investigated the occurrence of diseases in ripe tomatoes. The results showed that the occurrence of bacterial wilt, late blight and early blight, was much more pronounced in the crops grown with chemical fertilizer than in those grown with EM organic fertilizer. Other studies indicated that EM organic fertilizers increased the solubility of nutrients in the soil, promoted the formation and stability of soil aggregates, reduced soil compaction, accelerated soil organic matter, and increased the availability of nutrients in the soil, which facilitates nutrients' absorption and promotes plants' growth [Aoyama et al. 1999, Tian et al. 2001b, Celik et al. 2004, Mikha and Rice 2004, Johnson et al. 2006, Fares et al. 2008, Mosaddeghi et al. 2009].

Straw mulching means that the surface of the soil is covered with material, such as stubble straw (corn, wheat, or rice), leaves and weeds. Straw mulching is a type of crop management measure that can have a variety of effects, including water retention, increased fertility, tempering, weed control, and anti-erosion. The effective combination of organic fertilizer and straw mulching can effectively control the soil water and salt status and promote crop yield [Vidyasagar 1997]. Straw mulching can promote soil microbial content, reduce soil surface water evaporation, increase soil organic matter and nutrient content, increase soil carbon fixation capacity, and improve soil structure and physical and chemical properties [Blanco-Canqui and Lal 2007, Liu et al. 2010]. Döring et al. [2005] have shown that long-term coverage of no-tillage increases organic nitrogen and has a positive effect on soil organic matter accumulation due to mineralization of nitrogen in the straw and biological nitrogen fixation at microbial propagation.

In this study, the tomato was grown under the organic production practices of EM bio-organic fertilizer and straw mulching to research the effects of organic cultivation on growth characteristics, quality, disease resistance, and soil physical and chemical properties of tomato. This study provides some reference and materials for the promotion of tomato organic production practices, and it also has important theoretical and practical significance for accelerating the development of green organic agriculture to realize the sustainable development of agriculture.

MATERIALS AND METHODS

Plant materials and treatments. The tested tomato cultivar 'Miaohong' was provided by the Japanese Institute of Natural Agriculture. Tomato seeds were sown on April 30, 2016. The seeds were washed with 1% sodium hypochlorite for 20 min and then soaked in sterile water for 24 h. The seeds of the same size (full maturity) were seeded in a Petri dish with two layers of sterile filter paper, germinated in the dark at 28°C. The germinated tomato seeds were seeded in a bowl containing a ratio of peat : vermiculite of 2 : 1, and then cultured in an intelligent incubator with a daytime temperature of 25 ±5°C for 16 h and a nighttime temperature of 18°C for 8 h. The tomato seedlings were planted in the field after they grew to have four true leaves on May 20, 2016.

EM bio-organic fertilizer was applied one week before tomato seedlings planted in the field (May 13, 2016). The application method was as follows: first, the application amount was 0.315 $kg \cdot m^{-2}$, spread fertilizer evenly over the field; second, the soil was turned over with the rototiller to make the soil and fertilizer mixed after fertilization; then, soil was flattened. Topdressing was applied when the first fruit of the tomato appeared during the fruit period (July 16, 2016), spread fertilizer evenly over the field and timely irrigation. The amount of fertilizer is 0.18 $kg \cdot m^{-2}$. Straw mulching was carried out after tomato seedlings planted in the field (May 22, 2016). The method of straw mulching was as follows: straw mulching throughout the whole growing period of the tomato at an application amount of 0.6 $kg \cdot m^{-2}$ and the straw was chopped into approximately 3-cm lengths and evenly spread on the surface of the soil.

The experiment was designed with four treatments, as shown in Table 1, and each had three replicates. There were 12 plots for test, each with an area of 18 m^2. Fifty tomatoes were planted in each plot, the line spacing was 1 m and the row spacing was 0.5 m, and there was a 2-m wide isolation zone between each plot. Each treatment had the same field management technical measures, and no hormones, auxin, drugs, or watering methods using furrow irrigation were used during the entire growth period of tomato.

Effects of organic cultivation pattern on tomato growth. In the above-mentioned organic production practices, the growth characteristics of tomatoes

Table 1. Different treatments

Treatment	Planting pattern 1	Planting pattern 2	Planting pattern 3	Control
Straw cover	+	–	+	–
EM biological Organic fertilizer	+	+	–	–

+ represents the straw cover or application of EM bio-organic fertilizer; – represents no straw cover or no application of EM bio-organic fertilizer

Table 2. Real-time PCR Primer Sequence

Name	Sequence (5'–3')	Length (bp)
LeCHI-F	GTCAAACATCTCACGAAACT	20
LeCHI-R	TGTCGCAACTAAATCAGG	18
Actin-F	GGAATGGGACAGAAGGAT	18
Actin-R	CAGTCAGGAGAACAGGGT	18

under different treatments were observed. The statistical indicators are shown below: plant height, stem diameter, inflorescence node, and yield. The chlorophyll content and photosynthetic indexes of tomato in each plot were measured at the following periods: seedling, blossom, fruit, expanding, and mature. Five tomato plants were randomly selected from each treated plot, and the same functional leaves were picked for measuring the content of chlorophyll. The chlorophyll content was measured by using a spectrophotometer and 95% ethanol [Lichtenthaler 1987]. The photosynthetic rate (Pn), stomatal conductance (Gs), intercellular CO_2 concentration (Ci), and transpiration rate (Tr) were measured by an LCPRO + portable photosynthesis analyzer during sunny hours from 9.00 am to 11.00 am. Ten tomato plants were randomly selected from each treated plot, and the third functional leaf on the plant was picked for measurement.

Effects of organic cultivation pattern on tomato quality. We measured the quality of the tomato fruit at the mature period. To measure the quality, four fruits of each treatment were harvested, and then a quarter of each fruit was chopped. Soluble protein was determined using the Coomassie brilliant blue colorimetric method; soluble sugar was determined using the anthrone method; free amino acid was determined using the ninhydrin colorimetric method; reducing sugar was determined using the 3,5-dinitrosalicylic acid colorimetric method; vitamin C was determined using 2,6-dichlorophenol indophenol titration [Georgé et al. 2005]; nitrate was determined using the salicylic acid-sulfuric acid colorimetric method; organic acid was determined using acid-base titration [Kelebek and Selli 2011]. Glucose, fructose, and sucrose content was determined by high performance liquid chromatography per the procedures used by Madore [1990] and the extraction method of Hong-Yan et al. [2003], and changes.

Effects of organic cultivation pattern on tomato disease resistance. The morbidity of tomatoes under different treatments was calculated at the following periods: seedling, blossom, fruit, expanding, and mature. We also determined the relative resistance indexes of tomatoes that under different treatment at each growing season. We took the tomato fresh leaves samples from each plot for determination, and repeated the sampling three times. The indicators that we measured include: activity of superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT); soluble protein and malondialdehyde (MDA) content; relative expression of the tomato chitinase gene *LeCHI* in leaves. SOD activity was measured by the nitrogen blue tetrazolium (NBT) method; POD activity was measured by the guaiacol method; CAT activity was measured by the UV absorption method; soluble protein content was measured by the Coomassie brilliant blue G-250 staining method; and MAD content was determined using the sulfur generation of barbituric acid (TBA) method. Total RNA was extracted from the samples using a Tianze Gene Plant RNA Extraction Kit (CAT # 3080, Tianze Genetic Engineering Co., Ltd.). The total RNA of the tomato leaves was reverse transcribed into first-strand cDNA using a sea-based kit (Cat. No. D0501, Hokkaido Biotech Co., Ltd.). The primer of tomato chitinase gene *LeCHI* and reference gene are shown in Table 2.

Effects of organic cultivation pattern on soil physical and chemical properties of tomato plantations. The soil samples were collected by a snake-like 5-point method in each treated plot before the tomato planting in 2014, and we collected the soil with same method after the tomatoes were harvested in 2015 and 2016. The fresh soil samples were air dried, and then the debris was removed. The samples were then crushed to less than 1 mm and subsequently, the soil physical and chemical properties were determined. Soil total nitrogen was determined by the semi-micro Kjeldahl method; soil total phosphorus was determined by $HClO_4$-H_2SO_4; potassium was determined by an NaOH melting-flame photometer; the alkaline nitrogen was determined by the alkali dissolution method; the available phosphorus was determined by the $NaHCO_3$ extraction-molybdenum antimony anti-colorimetric method; the available potassium was determined by ammonium acetate extraction and flame spectrophotometry; soil organic matter was determined by the potassium dichromate volumetric method (dilution heat method); and soil pH was determined by a pH meter at a soil to water ratio of 1 : 2.5.

Statistical analysis

The results of this experiment were come from one vegetation period. The statistical analysis of the test data was performed using Microsoft Office Excel and SPSS 17.0 software.

RESULTS

Effects of organic cultivation pattern on tomato growth. Plant height and stem diameter are an indicator of plant growth and development, and they often reflect the robustness of plants. The effect of organic cultivation on the plant height and stem diameter of tomatoes during the different growth periods is shown in Figure 1. At the seedling stage, there was no significant difference in the pattern of each planting compared with the control group. At other stages, the plants under organic cultivation showed higher plant height and stem diameter than the control group; the overall trend was: plant pattern 1 > planting pattern 2 > planting pattern 3, and significant differences were seen. In the organic production practices, the first inflorescences of the tomato plants were reduced (Tab. 3). This phenomenon indicated that the flower bud differentiation of tomato was advanced and the vegetative organs were well developed. The effects of organic cultivation on tomato fruit weight, fruit number per plant, and yield are shown in Table 4. The results showed that the total fruit yield of organic tomato, the number of fruit per plant, and the overall trend of yield were: planting pattern 1 > planting pattern 2 > planting pattern 3 > blank control. Compared with the single application of EM bio-organic fertilizer, the EM bio-organic fertilizer and straw-covered organic production practices significantly increased single fruit weight, fruit number per plant, and yield.

Effects of organic cultivation pattern on tomato photosynthetic characteristics. Chlorophyll is the main pigment of photosynthesis of plants, which is one of the important indexes that reflect the changes in leaf physiological activity. It can be seen in Figure 2 that the chlorophyll content during the tomato growth period showed a single peak change, the chlorophyll content gradually increased during the seedling period to the expanding period, and then the chlorophyll content decreased slowly. Straw mulching or application of EM bio-organic fertilizer can significantly improve the chlorophyll content of tomato. The chlorophyll content of tomato in planting pattern 1 was significantly higher than that of each group during the fruit period to the mature period. The photosynthetic rate, stomatal conductance, transpiration rate, and intercellular CO_2 concentration of tomato plant leaves are shown in Figure 3. The results of the determination of the indicators show that the overall trend was: planting pattern 1 > planting mode 2 > planting mode 3 > blank control. In the organic production practices, the photosynthetic capacity of the tomato plants was improved, and planting pattern 1 showed a more remarkable effect.

Effects of organic cultivation pattern on tomato quality. The effect of organic cultivation on tomato quality is shown in Table 5. The results showed that the indexes of tomato fruit quality were improved under the organic production practices. There was no significant difference in soluble protein content between the treatments, but the soluble protein content was the highest under planting pattern 1, and the soluble protein content of the blank control group was the lowest. The soluble sugar content of pattern 1 was significantly higher than that of each group, and the soluble sugar content of the blank control was significantly lower than that of each group. The content of reducing sugar in planting pattern 1 was significantly higher than that in each group followed by planting pattern 2. Planting pattern 1 and planting pattern 2 significantly increased the content of reducing sugar; compared with the blank control, the reducing sugar content was increased by 22.7 and 10.1%, respectively. The content of free amino acids in each treatment group was not significant, but the content of free amino acid was the highest under planting pattern 1. The content of vitamin C in planting pattern 1 was the highest. Compared with the blank control, the vitamin C content was increased by 27.6%. The nitrate content of the blank control was the highest, whereas the nitrate content of planting pattern 1 and the nitrate content of planting pattern 2 were significantly lower than the blank control, and decreased by 26.9 and 16.8%, respectively, compared with the blank control.

Effects of organic cultivation pattern on tomato disease resistance. In order to evaluate the disease resistance of tomatoes in the organic production practices, we counted the incidence of tomatoes in each period, and the results are shown in Table 6. At the seedling stage and blossom stage, there were no diseased plants in any of the treated plots. In the fruit period, planting pattern 3 and blank control plot appeared to have diseased plants. At the expanding period, the morbidity of plants in each plot began to raise, the incidence of the blank control area reached 34.67%, while the

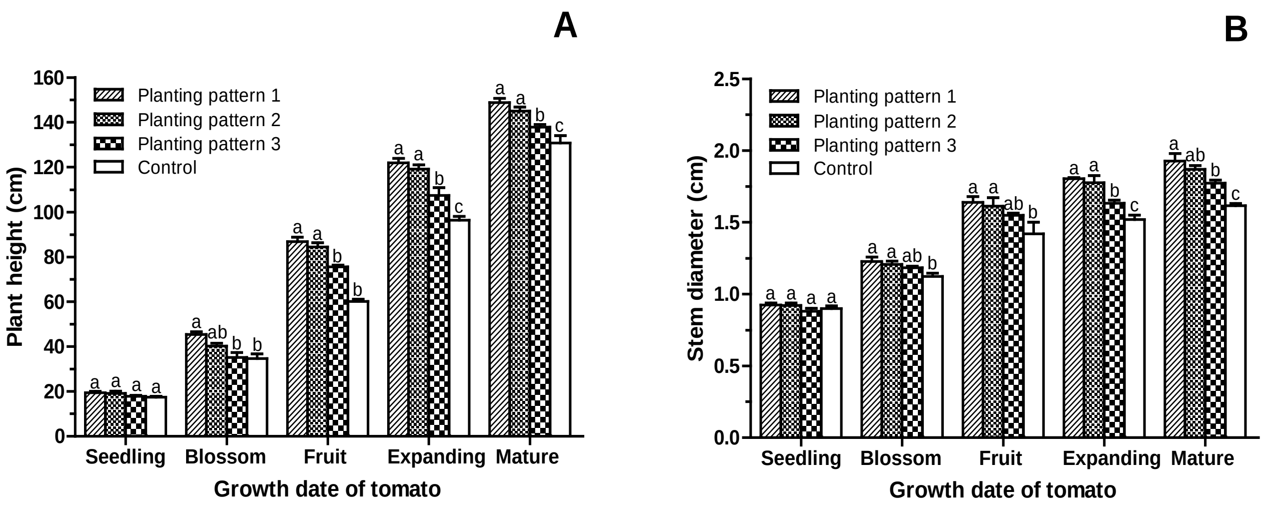

Fig. 1. Effect of organic cultivation on the plant height and stem diameter of tomatoes in the period of different growth dates: (A) plant height and (B) stem diameter. Data marked with different lowercase letters indicated that significant differences exist ($P < 0.05$) between different planting patterns in the same period

Table 3. Effects of organic production practicess on organic tomato inflorescence node

Treatment	First inflorescence node	Second inflorescence node	Third inflorescence node
Planting pattern 1	7–9	10–13	16–18
Planting pattern 2	7–9	11–13	17–19
Planting pattern 3	7–9	11–13	19–20
Control	8–10	13–14	19–21

Table 4. Effects of organic production practicess on organic tomato yield

Treatment	Weight of single (kg)	Fruit number per plant	Yield per 667 m^2 (kg)
Planting pattern 1	0.218 ±0.01a	21.67 ±1.15a	8480.7 ±153.42a
Planting pattern 2	0.209 ±0.01a	20.00 ±1.00a	7743.5 ±94.23b
Planting pattern 3	0.189 ±0.01b	19 ±0.93b	6811.5 ±200.31c
Control	0.187 ±0.01b	18 ±1.15b	6335.5 ±103.34c

Data marked with different lowercase letters indicated that significant differences exist ($P < 0.05$) between different planting patterns

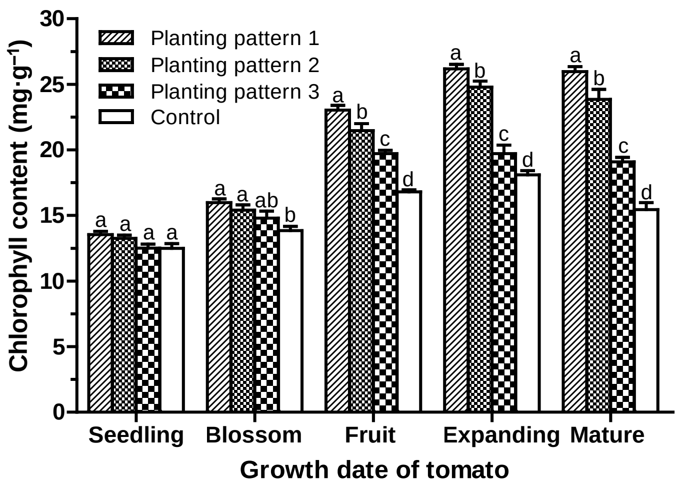

Fig. 2. Effect of organic cultivation on the chlorophyll content of tomatoes in the period of different growth dates. Data marked with different lowercase letters indicated that significant differences exist ($P < 0.05$) between different planting patterns in the same period

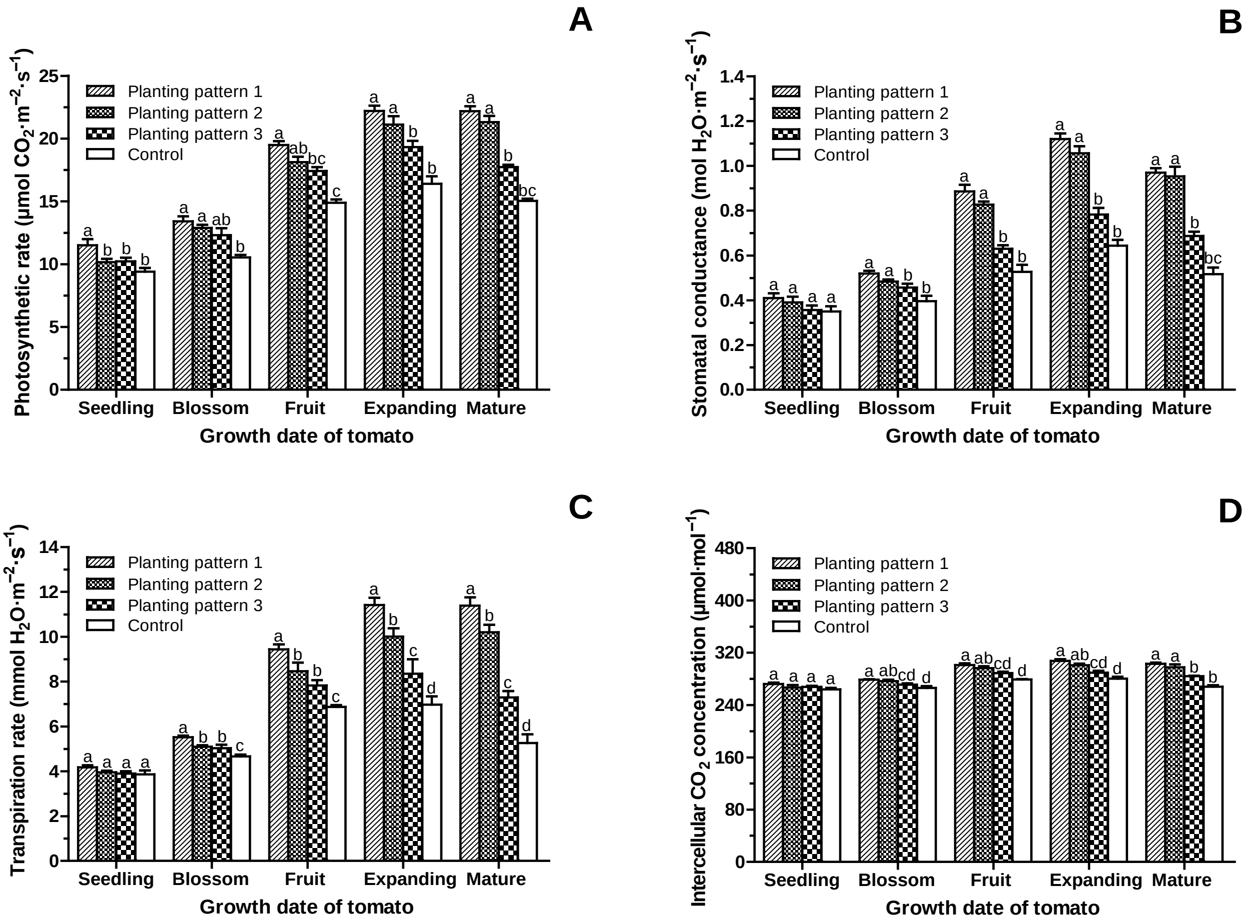

Fig. 3. Effect of organic cultivation on the photosynthetic capacity of tomatoes in the period of different growth dates: (A) photosynthetic rate, (B) stomatal conductance, (C) transpiration rate, and (D) intercellular CO_2 concentration. Data marked out with different lowercase letters indicated that significant differences exist (P < 0.05) between different planting patterns in the same period

Table 5. Effects of organic production practicess on organic tomato quality

Treatment	Soluble protein content (μg·100 g^{-1} FM)	Soluble sugar content (g·100 g^{-1})	Reducing sugar (mg·100 g^{-1})	Free amino acid (μg·100 g^{-1})	Vitamin C (mg·kg^{-1})	Organic acid (%)	Nitrate (mg·g^{-1})	Fructose (mg·g^{-1})	Glucose (mg·g^{-1})	Sucrose (mg·g^{-1})
Planting pattern 1	790.76 ±11.38a	3.07 ±0.20a	23.09 ±0.65a	42.42 ±1.74a	127.68 ±2.57a	0.50 ±0.02a	0.26 ±0.03c	11.57 ±0.86a	10.46 ±0.80a	0.43 ±0.02a
Planting pattern 2	774.00 ±4.51a	2.85 ±0.15b	20.73 ±1.35b	42.01 ±1.12a	116.85 ±7.47b	0.54 ±0.04a	0.27 ±0.03bc	10.77 ±0.65a	9.53 ±0.69ab	0.41 ±0.04a
Planting pattern 3	763.36 ±10.50a	2.73 ±0.15b	18.87 ±1.46c	39.68 ±1.42a	108.03 ±2.56bc	0.54 ±0.05a	0.32 ±0.05ab	9.23 ±0.36b	8.70 ±0.41bc	0.36 ±0.04b
Control	727.05 ±8.89a	2.35 ±0.13c	18.82 ±0.85c	38.69 ±0.48a	100.84 ±2.27c	0.57 ±0.06a	0.33 ±0.03a	8.91 ±0.31b	8.33 ±0.42c	0.34 ±0.03b

Note: Data marked with different lowercase letters indicated that significant differences exist (P < 0.05) between different planting patterns incidence of planting pattern 1 was 22.67%. At the mature stage, the incidence of tomato plants were seriously affected by disease invasion, but the incidence of planting pattern 1 was lowest at only 60%; the incidence of the blank control was 84.67%. The tomato disease often occurs during the fruit period, and it is serious in the period of expanding and mature. However, the application of EM bio-organic fertilizer has played a positive role in the resistance of tomato.

We also determined the soluble protein content (Fig. 4A), SOD (Fig. 4B), POD (Fig. 4C), CAT (Fig. 4D) activity, MDA content (Fig. 4E), and relative expression of the *LeCHI* gene in the leaves of tomato plants under different treatments (Fig. 5). During the blossom period to the expanding period, the soluble protein content of each treatment increased, but the amplitude was different. At planting pattern 1, the content of soluble protein in tomato leaves was the highest. Tomatoes that use EM bio-organic fertilizers can maintain a high soluble protein content for osmotic regulation against disease. On the whole, the activities of the three enzymes (SOD, POD, and CAT) of tomatoes were increasing from the seedling period to the mature period; under planting patterns 1 and 2, the three enzyme activities were at a high level and were significantly different from those of the other treatments. The results showed that the application of EM organic fertilizer and straw mulching could significantly improve the activities of antioxidant enzymes from the seedling period to the mature period, the MDA content in the leaves of each treatment showed an increasing trend, and the blank control was the most significant (Fig. 4E). Planting pattern 1 and planting pattern 2 maintained lower MDA content at all times.

Chitinase can hydrolyze the main components of the fungal cell wall, which fights against gray mold and other fungal diseases. Therefore, the expression of the chitinase gene can reflect the ability of plants to resist disease [Kovács et al. 2013]. The expression of the *LeCHI* gene in tomato leaves was analyzed by the fluorescence quantitative method (Fig. 5). From the fruit period to mature period, the expression level of the *LeCHI* gene in the treatment group increased first and then decreased, and the expression level of the *LeCHI* gene was the highest in the expanding stage. The expression level of this gene in planting pattern 1 was significantly higher than that of other treatment groups from the expanding period to the mature period, and the expression level of the *LeCHI* gene in the control group was significantly lower than that in planting pattern 1 and planting pattern 2.

Effects of organic cultivation patterns on soil physical and chemical properties. The effects of organic cultivation on soil physical and chemical properties are shown in Table 7. The data from 2014 is the basic fertility of the field soil. Total nitrogen is one of the indicators to measure the basic fertility of soil. In 2014, the total nitrogen content of soil was 1.72 $mg \cdot kg^{-1}$. The total nitrogen content of planting pattern 1 and planting pattern 2 in 2016 was significantly increased compared with that in 2014. The total nitrogen content of planting pattern 3 and the blank control in 2015 and 2016 were not significantly different from those in 2014, respectively. In 2014, the total phosphorus content of soil was 169.2 $mg \cdot kg^{-1}$, and the total phosphorus content of planting pattern 1 in 2016 was significantly higher than that in 2014, and increased by 16.7%. In 2015, the alkaline nitrogen content of planting pattern 1 increased by 22.6% compared with that in 2014, and this figure rose to 53.4% in 2016. Compared with 2014, the alkaline nitrogen content of planting pattern 2 increased by 19.1% in 2015, and this figure changed to 19.7% in 2016. In 2014, the available phosphorus in the soil was 20.12 $mg \cdot kg^{-1}$, and the available phosphorus content in planting pattern 1 in 2015 and 2016 was significantly increased by 22.37 and 36.33%, respectively, compared with that in 2014. In 2014, the available potassium content of soil was 169.2 $mg \cdot kg^{-1}$. Compared with 2014, the available potassium content of planting pattern 1 and planting pattern 2 in 2016 increased significantly. All three planting patterns increase the amount of organic matter in the soil. In 2014, the pH of the soil was 7.5, which means it was partial alkaline soil. The soil pH of planting pattern 1 was significantly lower in 2016 compared to 2014. Based on the above data, the application of EM organic fertilizer can significantly increase the content of total nitrogen, available phosphorus, and available potassium in soil. However, the combination of EM bio-organic fertilizer and straw mulching significantly increased the soil total phosphorus content and adjusted the soil pH to a certain extent.

Table 6. Effects of organic production practicess on the disease incidence of each cell of tomato (%)

Treatment	Seedling period	Blossom period	Fruit period	Expanding period	Mature period
Planting pattern 1	0	0	0	22.67 ±3.06	60.00 ±2.31
Planting pattern 2	0	0	0	26.00 ±2.00	66.33 ±2.00
Planting pattern 3	0	0	1.67 ±0.58	28.67 ±1.15	73.33 ±1.15
Control	0	0	3.33 ±1.15	34.67 ±1.15	84.67 ±2.00

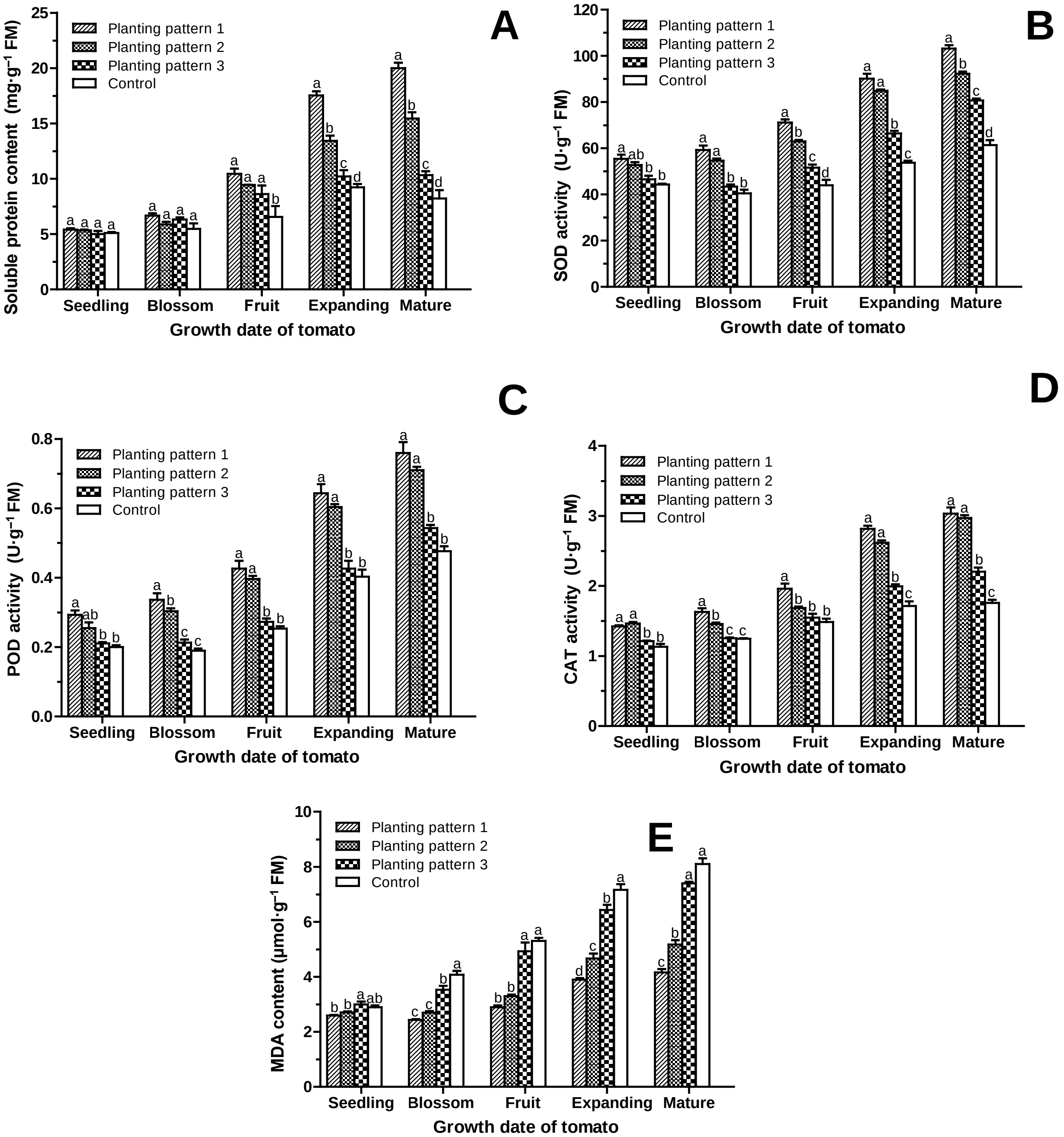

Fig. 4. Effect of organic cultivation on the physiological characteristics of tomatoes disease resistance. (A) Soluble protein content, (B) SOD activity, (C) POD activity, (D) CAT activity, and (E) MDA content. Data marked with differentlowercase letters indicated that significant differences exist ($P < 0.05$) between different treatments in the same period

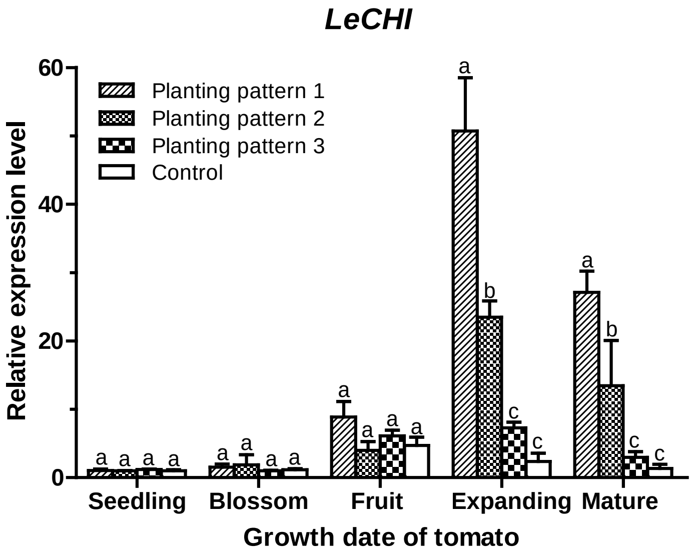

Fig. 5. Relative expression of the *LeCHI* gene in leaves of tomato plants under different planting patterns. Expression data correspond to means of triplicates, normalized to *actin*, and uses a control sample from seedling as the calibrator. Expression of data is in arbitrary units ±SE. Data marked with different lowercase letters indicate that significant differences exist ($P < 0.05$) between different treatments in the same period

Table 7. Effects of organic production practices on the soil physicochemical properties

Treatment	Year	Total nitrogen ($g \cdot kg^{-1}$)	Total phosphorus ($g \cdot kg^{-1}$)	Total potassium ($g \cdot kg^{-1}$)	Alkaline nitrogen ($mg \cdot kg^{-1}$)	Available phosphorus ($mg \cdot kg^{-1}$)	Available potassium ($mg \cdot kg^{-1}$)	Organic matter ($g \cdot kg^{-1}$)	Soil pH
Planting pattern 1	2014	1.72 ±0.63b	0.4 ±0.02b	15.12 ±2.46a	167.89 ±3.51c	20.12 ±2.03b	169.20 ±2.35b	18.10 ±1.74b	7.50 ±0.06a
	2015	2.1 ±0.63ab	0.45 ±0.03ab	17.03 ±1.03a	217.02 ±3.24b	24.62 ±2.34a	202.21 ±4.32a	23.77 ±2.12a	7.37 ±0.15ab
	2016	2.23 ±0.14a	0.48 ±0.02a	17.26 ±0.99a	258.67 ±0.51a	27.43 ±3.01a	213.17 ±6.61a	27.72 ±3.13a	7.31 ±0.25b
Planting pattern 2	2014	1.72 ±0.63b	0.4 ±0.02a	15.12 ±2.46a	167.89 ±3.51c	20.12 ±2.03b	169.20 ±2.35b	18.10 ±1.74b	7.50 ±0.36a
	2015	1.99 ±0.12ab	0.42 ±0.06a	15.71 ±1.43a	200.34 ±4.23b	23.48 ±3.43ab	192.20 ±5.32a	22.42 ±1.02a	7.42 ±0.15a
	2016	2.06 ±0.62a	0.45 ±0.07a	16.06 ±2.34a	237.33 ±3.79a	25.34 ±2.52a	201.67 ±6.91a	25.03 ±1.73a	7.37 ±0.75a
Planting pattern 3	2014	1.72 ±0.19a	0.4 ±0.02a	15.12 ±2.46a	167.89 ±3.51b	20.12 ±2.03a	169.2 ±2.35a	18.10 ±1.74b	7.50 ±0.36a
	2015	1.8 ±0.73a	0.41 ±0.03a	15.35 ±3.12a	188.88 ±0.68a	21.51 ±4.23a	178.36 ±5.34a	20.45 ±1.04ab	7.42 ±0.56a
	2016	1.81 ±0.23a	0.43 ±0.02a	15.75 ±2.34a	201.01 ±5.00a	22.32 ±2.52a	182.00 ±5.29a	21.38 ±1.88a	7.34 ±0.45a
Control	2014	1.72 ±0.63a	0.4 ±0.02a	15.12 ±2.46a	167.89 ±3.51a	20.12 ±2.03a	169.20 ±2.35a	18.10 ±1.74a	7.50 ±0.36a
	2015	1.79 ±0.64a	0.4 ±0.01a	15.45 ±2.23a	175.75 ±4.52a	21.14 ±1.52a	177.19 ±3.35a	19.01 ±1.34a	7.47 ±0.25a
	2016	1.79 ±0.57a	0.4 ±0.04a	15.693 ±2.43a	178.67 ±5.02a	21.99 ±5.03a	180.33 ±5.51a	19.27 ±1.50a	7.40 ±0.74a

Data marked with different lowercase letters indicate that significant differences exist ($P < 0.05$) between different years in the same planting patterns

DISCUSSION

There are many beneficial microorganisms in EM bio-organic fertilizers, such as photosynthetic bacteria and yeasts. The interaction between beneficial microorganisms can not only promote tomato root growth and cell division, but also produce hormones that promote the growth and development of tomato and increase the its nutrient content [Roe 1998, Bezborodov et al. 2010, Jordán et al. 2011]. Planting pattern 1 and planting pattern 2 significantly increased the plant height (Fig. 1A), stem diameter (Fig. 1B), fruit weight, and number of fruit per plant and yield of tomato (Tab. 4) compared with the blank control. EM microbial agents decompose organic matter in crop stalks into effective soluble substances, such as amino acids, sugars, and ethanol, which can be directly absorbed by the roots of plants to promote the growth and development of tomatoes. Chlorophyll is an important base material for photosynthesis. The higher the chlorophyll contents in plants, the stronger the potential photosynthetic capacity [Moradi and Ismail 2007]. The strength of photosynthetic capacity is closely related to plant growth, yield, and disease resistance [Nakano et al. 1997]. The chlorophyll content and photosynthetic index of planting pattern 1 and planting pattern 2 were significantly higher than those of the conventional treatment, indicating that the application of EM bio-organic fertilizer had a significant effect on chlorophyll content (Fig. 2) and photosynthetic index. Sugar species and content are the key to fruit quality, and soluble sugars, which are important indicators of quality traits, mainly contain fructose, glucose, and sucrose [Wegener et al. 2009]. Soluble sugar is the main product of higher plant photosynthesis, and is involved in carbohydrate metabolism and is also the main form of carbohydrate storage. Soluble sugar is also an osmotic regulator of many plants [Thorburn et al. 2011]. The results (Tab. 5) indicated that EM bio-organic fertilizer significantly increased the content of soluble sugar, reducing sugar, vitamin C, fructose, glucose, and sucrose in tomato, and decreased the content of nitrate. When EM bio-organic fertilizer is applied, it provides a variety of organic nutrients and trace elements for tomato, so that tomato plants become stronger. It also promotes the transport of nutrients to vegetative organs. The application of EM organic fertilizer is beneficial to the balance of carbon and nitrogen metabolism in tomato, which plays an important role in improving tomato development and quality, and also reduces the harm of agricultural products caused by excessive application of chemical fertilizer. The results of Thorburn et al. [2011] show that the rational application of bio-organic fertilizers can regulate sugar metabolism in crops.

Free radicals are harmful compounds produced in the oxidation reaction of plants. Under normal conditions, the production and removal of reactive oxygen in the plant maintain a balance. However, in the course of plant disease, the dynamic equilibrium is destroyed; a large number of oxygen free radicals can cause membrane lipid peroxidation, so that the plant tissue and cells are damaged [Fan et al. 2007]. Tomato disease begins to occur during the fruit period and becomes severe from the expanding period to the mature period. Compared with the blank control, tomatoes maintained a higher soluble protein content and higher SOD activity, POD activity, and CAT activity and a lower MDA content with the application of EM bio-organic fertilizers (Fig. 4). The application of EM bio-organic fertilizer could prevent the disease, and the expression of the chitinase *LeCHI* gene was significantly increased in the application of EM organic fertilizer and the straw-covered organic production practices compared with the single application of EM bio-organic fertilizer. In the process of degrading straw and other organic matter, EM organisms will produce a large number of anti-disease factors to kill or inhibit pests [Shaheen et al. 2014].

Straw mulching provides a favorable environment for tomatoes by regulating the ecological and environmental factors, such as water, fertilizer, gas, and heat in the soil [Tu et al. 2006]. Compared with the blank control, planting pattern 3 significantly increased the plant height (Fig. 1A) and stem diameter (Fig. 1B) of the tomatoes, but there was no significant effect on fruit weight, fruit number per plant, and yield (Tab. 4). There are many reports on the increase in yield of straw mulching, and the increase in yield is also closely related to the coverage of straw [Döring et al. 2005].

The coverage of straw was 0.6 $kg \cdot m^{-2}$ in this test, and the yield of tomato in the single straw-covered mode was 7.5% higher than that of the blank control. The suitable straw mulching for planting tomato could be further optimized. Planting pattern 1 and planting pattern 3 significantly increased the chlorophyll content (Fig. 2) and transpiration rate (Fig. 3C) of tomato. Zhao et al. [2002] found that straw mulching could increase the photosynthesis and chlorophyll content of apple pear leaves in arid desert areas. The rate of transpiration directly affects the amount of soil moisture content. Straw mulching indirectly affected the transpiration rate of leaves by affecting the content of soil moisture, which may be the reason why the transpiration rate of planting pattern 3 was higher than that of the blank control (Fig. 3C). Compared with the blank control, the soluble sugar content (Tab. 5) of tomato in planting pattern 3 increased significantly, by 16.2%, but had no significant effect on other quality indexes.

Fertile loose soils provide a relatively stable matrix environment for crop growth and development. The beneficial microorganisms in the bio-organic fertilizer have the ability to dissolve phosphorus and potassium and fix nitrogen, enrich and balance the soil nutrients, improve the soil micro-ecological environment, and accelerate the decomposition of soil organic matter and nitrogen, phosphorus, and potassium nutrients [Schlegel et al. 1992, Egashira et al. 2003]. Straw mulching can not only increase the soil organic matter, total nutrients, and available nutrient content, but also increase soil microbial species and quantity, improve soil structure and physicochemical properties, and reduce the nutrient loss of soil surface. Under the appropriate environmental conditions, the combination of straw mulching and EM bio-organic fertilizers allows the release of various nutrient elements to provide excellent growth conditions for the roots of the plant. The results showed that the effect of single application of bio-organic fertilizer or single straw mulching on the pH value was not significant, and the organic production practices of the EM bio-organic fertilizer and straw mulching significantly reduced the soil pH value (Tab. 7). When the test soil is alkaline soil, under the appropriate temperature and anaerobic environment, the EM biocide will draw most of the lignocellulosic material into sugar and other organic compounds. Carbohydrates are converted into lactic acid and volatile fatty acids by organic acid fermentation, which play an important role in regulating soil pH. It can be seen that the combination of bio-organic fertilizer and straw mulching has a potential value for the development and utilization of saline soil.

CONCLUSION

Compared with the traditional production practices, the organic production practices has many advantages. EM bio-organic fertilizer or straw mulching can significantly improve the growth status, quality, and disease resistance of tomatoes, and it also plays an important role in improving soil quality.

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