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Case Reports

Effects of Foliar Applications Mulberry Leaf Enzyme Solution Fertilizer on Growth and Quality of Hydroponic Chinese Cabbage

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Abstract: To investigate the effects of foliar applications mulberry leaf enzyme solution fertilizer on growth, nutritional quality, photosynthetic characteristics, mineral elements, and antioxidant enzyme system of hydroponic Chinese cabbage, Chinese cabbage Zheng bai'886'was used as experimental material. In this study, we mainly selected seven different fermentation periods (CK: Distilled water, T1-T6, the fermentation days were 20, 35, 50, 65, 80, and 95 d) of mulberry leaf enzyme solution as foliar fertilizer on the effects of hydroponic Chinese cabbage. The results showed that plant height, plant width, leaf area, maximum root length, fresh and dry weight, soluble sugar, soluble protein, Vc, anthocyanin content, and photosynthetic capacity of Chinese cabbage were significantly increased compared with CK when spraying fermented for 50 d mulberry leaf enzyme solution and nitrate nitrogen content in Chinese cabbage decreased significantly. Compared with CK, the accumulation of calcium, magnesium, and iron in the shoot increased by 56.58, 50.70 and 36.67%, respectively, and antioxidant enzyme activities (SOD, CAT, POD) of Chinese cabbage was improved by spraying fermented for 50 d mulberry leaf enzyme solution. In general, sprayed fermented for 50 d mulberry leaf enzyme solution has a relatively good effect on hydroponic Chinese cabbage.

Keywords: Mulberry Leaf Enzyme Solution, Foliar Fertilization, Hydroponic Chinese Cabbage, Growth, Quality

Introduction

With the improvement of our people's living standards and the rapid economic development, people have higher and higher requirements for the quality of safe, green, and pollution-free vegetables. How to cultivate vegetables with good shape and high quality has been widely concerned by researchers. Among them, plant enzyme nutrient solution is an effective method that can significantly promote the nutritional growth and quality of vegetable crops. The plant enzyme solution is composed of plant residues, brown sugar, and water (Zhang *et al*., 2018). Fruits and vegetables are excellent substrates for fermentation due to their high content of carbohydrates, polyphenols, vitamins, minerals, and dietary fibers (Szutowska, 2020), such as potatoes, carrots, pomegranates, cashew apples, cranberries, jujube, and noni fruits, etc., can be used to produce fermented vegetables. In addition, hydroponic systems lead to a great reduction of inputs, such as water and fertilizers are suitable for the growth of leafy vegetables even without the use of substrates (Tsouvaltzis *et al*., 2020). Mulberry leaf has been identified as an excellent resource with high content of protein, carbohydrates, vitamins, microelements, and dietary fiber (Yu *et al*., 2018) and also contains rutin, quercetin, isoquercetin and other flavonoids (Chauhan *et al*., 2013), it plays an important role in human health, including antioxidant, anticancer, antidiabetic and immunomodulatory effects (Memete *et al*., 2022). Mulberry resource is rich in our country, which produces a large number of mulberry leaves every year. In sustainable animal production systems, the mulberry leaf is only used for sericulture in almost every part of the world (Srivastava *et al*., 2003), it has been recognized as a suitable alternative for supplementing livestock diets (González-García and Martín Martín, 2017). However, in plant production, there are few reports of using the mulberry leaf as raw materials to ferment to produce an organic nutrient solution for crop cultivation, the use of mulberry leaf is very simple so a large number of mulberry leaves waste. This experiment sprayed different periods of mulberry leaf enzyme solution hydroponic Chinese cabbage, studied the effects of foliar applications mulberry leaf enzyme solution fertilizer on the growth and quality of hydroponic Chinese cabbage, and explore the feasibility of using the mulberry leaf as a resource to develop plant organic nutrient solution, to provide a reference for further improving mulberry leaf in development and utilization, resource utilization rate and development and utilization of plant organic nutrient solution.

Materials and Methods

Experimental Sites and Materials

This field experiment was conducted in the plastic greenhouse in the third living area of Henan Agricultural University from May to July 2021; the related index tests were carried out in the laboratory of the Facilities Department of the College of Horticulture, Henan Agricultural University from July to August 2021. The experimented Chinese cabbage seed variety is ‘Zheng bai 886’ (purchased from Zhengzhou Hardware Agricultural Technology Development limited company). Mulberry leaf enzyme solution was prepared in the laboratory and the whole fermentation process was completed from July to October 2020. The length of the fermented mulberry leaf is 2 ~ 3 cm. The preparation method is Mulberry leaf (w): Brown sugar (w): Distilled water (w) = 10:3:40 and the fermentation is carried out with this material ratio. It is prepared by combining aerobic and anaerobic fermentation methods: In the early stage of aerobic fermentation, stirred once a week, measured the temperature, EC, and pH value and took 50 mL of fermentation solution; at the later stage of fermentation, stirred once every half month and took samples. After 95 d of fermentation when EC and pH values stabilized, the filtrate was taken to complete the preparation of mulberry leaf enzyme solution, and relevant data were collected. The whole fermentation process was carried out in a dark environment covered with a black-and-white film, the nutrient composition and physicochemical properties of the mulberry leaf enzyme solution were shown in Table 1.

Experimental Treatments and Methods

Based on Preliminary experiments, the experiment used the mulberry leaf solution diluted 400 times as foliar fertilization. The experiment had a randomized complete block design with six replications and comprised the following fertilization treatments(Table 2): Control-spray distilled water (CK), mulberry leaf enzyme solution fermented for 20 d (T1), mulberry leaf enzyme solution fermented for 35 d (T2), mulberry leaf enzyme solution fermented for 50 d (T3), mulberry leaf enzyme solution fermented for 65 d (T4), mulberry leaf enzyme solution fermented for 80 d (T5), mulberry leaf enzyme solution fermented for 95 d (T6).

The experiment adopts the NFT seedling method, it was planted in the seedling layer (77 × 40 × 170 cm) of the "Oxygen vegetable source" intelligent vegetable planting machine (purchased from Zhengzhou Maijia Agricultural Technology limited company). The seeds are first sown into round holes in the 100-hole seedling sponge, then put the planting cotton in the holes of the 80-hole seedling layer, sprayed water to moisten the seeds and the nutrient solution used was a modified Hoagland formula. After 15 d, the seedlings were graded and the seedlings of the same size were randomly transplanted into the holes in the four planting layers. Hydroponic systems: A cultivation box with a volume of 50 L, including a 3 W water pump with "Oxygen vegetable source" and indoor intelligent vegetable planting machine exclusive DC 12 V solution level device; set the time of solution supply, light on and dark time and the control accuracy is 1 min. During the Chinese cabbage's growth period, always provided a nutrient solution. In addition, the seeds were protected from light before germination and photosynthesized from 7:00 to 19:00 every day after germination, providing oxygen for 15 min per hour. From 20:00 to 06:00, oxygen was provided for 15 min at 24:00 and 06:00, respectively, the rest of the time the seeds perform respiration. After 10 d of planting, the photosynthetic data were measured from 9:00 to 11:00 in the morning on a sunny day and then every 5 d for a total of 3 times; after 25 d of planting, six plants were randomly sampled from each group for relevant data collection.

Measurement Indexes and Methods

Six Chinese cabbages were selected randomly in the experiment. Use tape to measure the plant height (distance from the base of the stem to the growing point of the Chinese cabbage, unit: cm); Plant width (use a 50 cm ruler to measure the maximum distance between the spread of the leaves centered on the growth point, unit: cm); Number of leaves (not counted if the leaf length is less than 5 cm); Measure the maximum root length with a ruler; Dry and fresh weight above ground: Use the analytical balance directly to determine the ground weight as fresh weight, then 105°C for 15 min, 85°C constant temperature drying to constant weight, weighing as dry weight); Soluble sugar content was measured by anthrone colorimetry. Soluble protein content was measured by Corma's bright blue staining (Luo *et al*., 2012). Vc content was measured by 2, 6-D titration. Anthocyanin content was measured by the absorbance method (Zhang *et al*., 2018). Superoxide Dismutase (SOD) activity, Catalase (CAT) activity, Ascorbate Peroxidase (APX), and Peroxidase (POD) activity were determined according to the instructions of the kits (purchased from Beijing Solebo Technology limited company). Chlorophyll and carotenoid contents were measured by spectrophotometry (Li *et al*., 2000). Net photosynthetic rate (Pn, $umolCO_2/m^2 \cdot S$), transpiration rate (Tr, $mmolH_2O/m^2 \cdot S$), stomatal conductance (Gs, $molH_2O/m^2 \cdot S$), and intercellular CO_2 concentration (Ci, $umolCO_2/mol$) were determined by li-6400XT portable photosynthetic apparatus.

Statistical Analysis

Data were analyzed by SPSS 20.0 software, data statistics and graphs were used by Office 365 Excel software, and the LSD test method was used for multiple comparisons of significant differences (*P<0.05*).

Results and Analysis

Effects of Spraying Mulberry Leaf Enzyme Solution on the Morphology of Hydroponic Chinese Cabbage

The plant width, leaf numbers, leaf area, and root volume of hydroponic Chinese cabbage showed a trend of first increasing and then decreasing with the increase of mulberry leaf fermentation days (Table 3). Among them, the plant width of T3 was significantly higher than those of CK, which increased by 15.55% compared with CK, the difference between leaf numbers in all treatments and CK was not significant. The leaf area of all treatments was significantly higher than CK and the leaf area of T3 was the largest, which increased by 85.93%. No significant differences were detected in the leaf area between other treatments and CK. The difference between leaf numbers in all treatments and CK was not significant. The plant height and maximum root length of Chinese cabbage increased firstly, then decreased, then increased, and then decreased with the increase of mulberry leaf fermentation days. The plant height of T3, T5, and T6 was significantly higher than that of CK. The plant height of T3 was the largest and increased by 19.70% compared with CK, while the difference between other treatments and CK was not significant. The maximum root length of T2 was the largest and significantly higher than that of CK, which increased by 23.46% compared with CK. The maximum root length of T6 was significantly lower than that of CK and the difference between other treatments and CK did not reach a significant level. The results showed that sprayed mulberry leaf enzyme solution of different fermentation periods was beneficial to the aboveground growth of hydroponic Chinese cabbage and the maximum root length was inhibited when spraying fermented for 80 d mulberry leaf enzyme solution. In conclusion, sprayed fermented for 50 d mulberry leaf enzyme solution had the best effect on the morphology of Chinese cabbage.

Effects of Spraying Mulberry Leaf Enzyme Solution on Dry and Fresh Weight of Hydroponic Chinese Cabbage

The dry and fresh weights of hydroponic Chinese cabbage showed a trend of first increasing and then decreasing with the increase of mulberry leaf fermentation days (Table 4). Among them, the dry and fresh weights of T3 were the largest and were significantly higher than CK, which increased by 50.0, 72, 51.45, 67.76, 43.96, and 66.74% compared with CK, respectively. The results showed that spraying the mulberry leaf enzyme solution of different fermentation periods was beneficial to the growth of hydroponic Chinese cabbage and sprayed fermented for 50 d mulberry leaf enzyme solution had the best effect on dry and fresh weight of Chinese cabbage.

Effects of Spraying Mulberry Leaf Enzyme Solution on Photosynthetic Pigment Content of Hydroponic Chinese Cabbage

Chlorophyll a, chlorophyll b, and carotenoid contents in hydroponic Chinese cabbage first increased, then decreased, then increased, and then decreased with the increase of mulberry leaf fermentation days (Table 5). Chlorophyll content in T3, T4, and T5 was significantly higher than those in CK, among which T3 was the largest and increased by 52.94% compared with CK and the differences between other treatments and CK were not significant. There was no significant difference between chlorophyll b content and CK in all treatments. T3 has the highest carotenoid content, which was significantly higher than CK and increased by 20% compared with CK, while the difference in carotenoid content between other treatments and CK was not significant. Chlorophyll content of Chinese cabbage increased firstly and then decreased with the increase of mulberry leaf fermentation days and chlorophyll pigment content in all treatments was significantly higher than that in CK, among which chlorophyll pigment content in T3 was the largest and increased by 45.65% compared with CK. The results showed that sprayed mulberry leaf enzyme solution was beneficial to the accumulation of chlorophyll a, chlorophyll pigment, and carotenoid content in hydroponic Chinese cabbage, but had little effect on the accumulation of chlorophyll b content. In conclusion, sprayed fermented for 50 d mulberry leaf enzyme solution had the best effect on the improvement of photosynthetic pigment content in hydroponic Chinese cabbage.

Table 1: The nutrient composition and physicochemical properties of Mulberry leaf enzyme solution

Component content (mg/l)	Nitrogen content (mg/l)	Phosphorus content (mg/l)	Potassium content (mg/l)	Mg (mg/l)	Ca (mg/l)	Fe (mg/l)	Soluble sugar content (mg/l)	pH 值 value	EC 值 value (mS/cm)
CK	3846.67±58.33e	1785.05±03.05c	115.20±8.13g	9.13±0.44e	256.83±15.17d	41.93±0.43a	28832.92±00.24a	4.07±0.14a	2.90±0.21c
T1	5618.33±39.19d	2192.46±10.31b	614.07±2.85f	160.47±2.13a	293.37±03.17bcd	35.23±1.34b	4681.94±47.71b	2.90±0.02d	4.49±0.03a
T2	6151.67±58.33c	5676.92±07.44a	667.13±5.70d	131.60±4.97bc	186.10±03.29e	29.77±1.24c	3898.61±15.74c	3.53±0.03b	4.44±0.07ab
T3	7256.67±44.19a	2217.92±21.96b	826.73±2.27a	140.73±1.92b	296.13±06.51bc	28.13±2.15c	3743.75±20.45d	3.54±0.04b	4.24±0.19ab
T4	6740.00±02.88b	672.62±14.13f	769.00±6.00b	136.67±3.21b	327.47±06.26b	29.63±1.20b	3626.67±13.47e	3.10±0.02c	4.32±0.05ab
T5	6655.00±51.96b	1451.28±02.92e	698.40±2.69c	124.20±4.42c	270.97±05.69cd	31.50±2.25bc	3285.00±16.30f	3.05±0.04cd	3.84±0.34b
T6	7275.00±35.00a	1496.36±00.57d	650.10±3.52e	52.40±0.70d	450.07±24.97a	13.00±0.46d	2925.00±04.33g	3.03±0.04cd	4.09±0.23ab

Note: Values followed by different small letters indicate significant differences among treatments ($p \leq 0.05$), as the following table

Table 2: The fertilization treatments of mulberry leaf enzyme solution

Symbols	Treatments
CK	Spray distilled water
T1	Mulberry leaf enzyme solution fermented for 20 d
T2	Mulberry leaf enzyme solution fermented for 35 d
T3	Mulberry leaf enzyme solution fermented for 50 d
T4	Mulberry leaf enzyme solution fermented for 65 d
T5	Mulberry leaf enzyme solution fermented for 80 d
T6	Mulberry leaf enzyme solution fermented for 95 d

Table 3: Effects of spraying mulberry leaf enzyme solution on the morphology of hydroponic Chinese cabbage

Treatment	Plant height (cm)	Plant width (cm)	Leaf number (slices)	Leaf area (cm^2)	Maximum root length (cm)	Root volume (cm^3)
CK	21.47 ± 0.43^{d}	30.87 ± 0.79^{bc}	10.67 ± 0.33^{a}	2194.44 ± 16.81^{f}	27.03 ± 0.88^{bc}	2.67 ± 0.33^{ab}
T1	22.70 ± 0.42^{bcd}	31.00 ± 0.92^{bc}	11.00 ± 0.58^{a}	2844.72 ± 66.04^{d}	29.25 ± 1.88^{b}	3.33 ± 0.33^{a}
T2	22.23 ± 0.42^{cd}	33.40 ± 0.95^{ab}	11.67 ± 0.33^{a}	3055.50 ± 10.28^{c}	33.37 ± 1.78^{a}	3.33 ± 0.33^{a}
T3	25.70 ± 1.41^{a}	35.67 ± 1.07^{a}	12.00 ± 0.58^{a}	4080.22 ± 18.98^{a}	26.15 ± 0.49^{bcd}	3.67 ± 0.33^{a}
T4	23.40 ± 0.50^{bcd}	31.33 ± 1.70^{bc}	11.33 ± 0.33^{a}	2453.30 ± 80.60^{e}	25.00 ± 0.23^{cd}	2.67 ± 0.67^{ab}
T5	24.63 ± 0.24^{ab}	29.47 ± 1.09^{c}	11.67 ± 0.33^{a}	3157.90 ± 24.80^{bc}	24.17 ± 0.81^{cd}	2.67 ± 0.33^{ab}
T6	23.73 ± 0.58^{abc}	29.70 ± 1.25^{bc}	11.67 ± 0.33^{a}	3287.27 ± 77.47^{b}	22.80 ± 0.32^{d}	1.67 ± 0.33^{b}

Table 4: Effects of spraying mulberry leaf enzyme solution on dry and fresh-weight of hydroponic Chinese cabbage

	Fresh weight (g/plant)			Dry weight (g/plant)		
Treatment	Shoot	Root	Whole Plant	Shoot	Root	Whole Plant
CK	46.46 ± 2.27^{d}	2.07 ± 0.11^{bc}	48.53 ± 2.37^{d}	2.86 ± 0.09^{d}	0.25 ± 0.01^{c}	3.11 ± 0.10^{d}
T1	64.48 ± 2.43^{bc}	2.71 ± 0.41^{ab}	67.19 ± 2.10^{bc}	3.87 ± 0.17^{b}	0.41 ± 0.03^{a}	4.28 ± 0.19^{b}
T2	58.32 ± 0.96^{c}	2.57 ± 0.18^{ab}	60.89 ± 0.82^{c}	3.45 ± 0.14^{c}	0.29 ± 0.02^{bc}	3.74 ± 0.13^{c}
T3	77.94 ± 2.78^{a}	2.98 ± 0.08^{a}	80.92 ± 2.83^{a}	4.29 ± 0.16^{a}	0.43 ± 0.03^{a}	4.71 ± 0.17^{a}
T4	57.62 ± 2.84^{c}	1.75 ± 0.05^{c}	59.37 ± 2.83^{c}	2.91 ± 0.04^{d}	0.22 ± 0.03^{c}	3.13 ± 0.06^{d}
T5	61.35 ± 3.41^{c}	2.85 ± 0.29^{a}	64.19 ± 3.50^{c}	3.56 ± 0.06^{bc}	0.36 ± 0.03^{ab}	3.93 ± 0.04^{bc}
T6	71.94 ± 2.46^{ab}	2.68 ± 0.18^{ab}	74.62 ± 2.64^{ab}	3.87 ± 0.13^{b}	0.36 ± 0.03^{ab}	4.23 ± 0.11^{b}

Note： Values followed by different small letters indicate significant differences among treatments (*p≤0.05*)

Table 5: Effects of spraying mulberry leaf enzyme solution on photosynthetic pigment content (mg/g FW) of hydroponic Chinese cabbage

Treatment	Chlorophyll a content	Chlorophyll b content	Chlorophyll content	Carotenoid content
CK	0.34 ± 0.032^{c}	0.13 ± 0.009^{ab}	0.46 ± 0.039^{b}	0.10 ± 0.013^{bc}
T1	0.39 ± 0.007^{bc}	0.13 ± 0.003^{ab}	0.51 ± 0.010^{b}	0.10 ± 0.001^{abc}
T2	0.36 ± 0.017^{c}	0.14 ± 0.018^{ab}	0.50 ± 0.032^{b}	0.08 ± 0.003^{c}
T3	0.52 ± 0.015^{a}	0.16 ± 0.006^{a}	0.67 ± 0.012^{a}	0.12 ± 0.006^{a}
T4	0.48 ± 0.027^{ab}	0.14 ± 0.009^{ab}	0.62 ± 0.038^{a}	0.10 ± 0.003^{ab}
T5	0.46 ± 0.055^{ab}	0.15 ± 0.012^{ab}	0.61 ± 0.047^{a}	0.10 ± 0.005^{ab}
T6	0.35 ± 0.021^{c}	0.12 ± 0.01^{b}	0.46 ± 0.027^{b}	0.08 ± 0.006^{c}

Note: Values followed by different small letters indicate significant differences among treatments (*p≤0.05*)

Effects of Spraying Mulberry Leaf Enzyme Solution on Photosynthetic Characteristics of Hydroponic Chinese Cabbage

In sprayed mulberry leaf enzyme solution of different periods, the Photosynthetic rate (Pn) and Transpiration rate (Tr) of Chinese cabbage showed a trend of first increasing and then decreasing with the increase of planting days, the effect on stomatal conductance (Gs) and intercellular carbon dioxide Concentration (Ci) showed a trend of first decreasing and then increasing (Fig. 1). When planted 10 d, Pn of Chinese cabbage under T4-T6 were significantly reduced compared with CK, but there was no significant difference between other treatments and CK. When planted 15 d, compared with CK, Pn of T6 was significantly increased by 27.65% and there was no significant difference between other treatments and CK. When planted 20 d, there was no significant difference between Pn and CK of Chinese cabbage in all treatments (Fig. 1A). When planted 10 d, compared with CK, Gs of Chinese cabbage in T2, T5 and T6 were significantly reduced, while the difference between other treatments and CK were not significant, but there was no significant difference between other treatments and CK. When planted 15 d, compared with CK, T5 and T6 significantly reduced the Gs content of Chinese cabbage, but there was no significant difference between other treatments and CK. When planted 20 d, compared with CK, Gs of T5 and T6 were significantly higher than CK (Fig. 1B). When planted 10 d, compared with CK, Ci of T3 and T6 were significantly reduced and the difference between other treatments and CK were not significant. When planted 15 d, compared with CK, Ci of T3 and T6 increased by 12.95 and 14.18%, respectively. When planted 20 d, compared with CK, Ci of T5 and T6 increased significantly, while there was no significant difference between other treatments and CK (Fig. 1C). When planted 10 d, compared with CK, Tr of T3 and T4 were significantly higher than that of CK and T3 was the largest and increased by 37.78% compared with CK. When planted 15 d, compared with CK, Tr of T5 was significantly reduced and there was no significant difference between other treatments and CK. When planted 20 d, compared with CK, Ci of T6 was significantly lower than that of CK and there was no significant difference between other treatments and CK (Fig. 1D). The results show that sprayed the mulberry leaf enzyme solution of different fermentation periods can significantly improve the photosynthetic characteristics of hydroponic Chinese cabbage. When planted 10 d, T3 had the best promoting effect and the transpiration rate was significantly increased by 37.78% compared with CK. When planted 15 d, T6 had the best promoting effect and the net photosynthetic rate was significantly increased by 27.65% compared with CK. When planted 20 d, T3 had the best-promoting effect and the transpiration rate was significantly increased by 18.31% compared with CK.

Effects of Spraying Mulberry Leaf Enzyme Solution on Nutritional Quality of Hydroponic Chinese Cabbage

Soluble sugar, soluble protein, and anthocyanin contents in hydroponic Chinese cabbage showed a trend of first increasing and then decreasing with the increase of mulberry leaf fermentation days (Table 6). Soluble sugar contents of T1-T3 were significantly higher than that of CK, among which T3 was the largest and increased by 26.60% compared with CK, the differences between other treatments and CK did not reach a significant level. Soluble protein contents of all treatments were significantly higher than CK and the difference among treatments did not reach a significant level. The anthocyanin content of T3 was significantly higher than that of CK, which increased by 20.12% compared with CK, and the difference between other treatments and CK did not reach a significant level. Vc content of T3 and T5 were significantly higher than that of CK and the Vc content of T3 was the largest, significantly increasing by 23.86% compared with CK, while the difference between other treatments and CK did not reach a significant level. The nitrate nitrogen content of each treatment sprayed with mulberry leaf enzyme solution was significantly reduced. Among them, the Nitrate nitrogen of T3 was the lowest, which was 35.26% less than that of CK. The difference between other treatments and CK was not significant. The results showed that sprayed mulberry leaf enzyme solution within 50 d could significantly reduce nitrate nitrogen content in hydroponic Chinese cabbage and the longer the fermentation time, the less significant the reduction of nitrate nitrogen content. Based on the above indexes, spraying the mulberry leaf enzyme solution of different fermentation periods was beneficial to the accumulation of soluble sugar, Vc, and anthocyanin in hydroponic Chinese cabbage, but there was no significant effect on the accumulation of soluble protein. Moreover, sprayed fermented for 50 d mulberry leaf enzyme solution had the best effect on the nutritional quality of Chinese cabbage, which could not only reduce nitrate nitrogen content to the greatest extent but also improve the nutrient quality of hydroponic Chinese cabbage.

Effects of Spraying Mulberry Leaf Enzyme Solution on Mineral Element Accumulation of Hydroponic Chinese Cabbage

The growth and quality of plants are closely related to the accumulation of mineral elements. Sprayed mulberry leaf enzyme solution had a significant effect on mineral element accumulation in hydroponic Chinese cabbage (Table 7). In this experiment, compared with CK, the total nitrogen content of each treatment did not reach a significant level. The total phosphorus content of T1 was significantly higher than that of CK. Except for T2 and T5, the total potassium content of all treatments was significantly higher than CK. The addition of mulberry leaf enzyme solution was beneficial to the accumulation of calcium, magnesium, iron, and zinc in the above-ground of hydroponic Chinese cabbage. Among all the treatments, the content of T3 was the highest, which significantly increased by 56.58, 50.70, 36.67, and 8% compared with CK (Table 7). The accumulation of mineral elements in the root of hydroponic Chinese cabbage increased first and then decreased. The total nitrogen content of T1-T3 was significantly higher than CK. Except for T2 and T3, total phosphorus content was significantly lower than CK and other treatments had no significant difference compared with CK. The total potassium content of each treatment was significantly higher than CK. The calcium content of each treatment was significantly lower than CK. The iron content of T4 was significantly lower than that of CK and there was no significant difference in the iron content of each treatment. Except that there was no significant difference in zinc content between T2 and CK and other treatments were significantly lower than CK. In conclusion, sprayed fermented for 50 d mulberry leaf enzyme solution had the

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best effect on the improvement of photosynthetic pigment content in hydroponic Chinese cabbage.

Effects of Spraying Mulberry Leaf Enzyme Solution on the Content of Malondialdehyde of Hydroponic Chinese Cabbage

Malondialdehyde content in hydroponic Chinese cabbage showed a trend of first decreasing and then increasing. Malondialdehyde content of T3 and T6 were significantly lower than CK, which was 27.95 and 42.05% lower than CK, respectively, the difference between other treatments and CK did not reach a significant level (Fig. 2). The results showed that sprayed the mulberry leaf enzyme solution of different fermentation periods had a certain effect on the reduction of malondialdehyde content in hydroponic Chinese cabbage and sprayed fermented for 95 d mulberry leaf enzyme solution had the most significant effect on the reduction of malondialdehyde content.

Effects of Spraying Mulberry Leaf Enzyme Solution on the Antioxidant Enzyme System of Hydroponic Chinese Cabbage

Sprayed mulberry leaf enzyme solution of different periods, which could improve the antioxidant enzyme activities (SOD, CAT, POD) of hydroponic Chinese cabbage (Fig. 3). SOD activity of T1-T3, CAT activity of T3, T4 were significantly higher than that of CK and SOD, CAT and POD activities of T3 were the largest, which increased by 23.09, 46.53 and 50.33% compared with CK, respectively and the differences between the indexes of other treatments and CK did not reach a significant level. The results showed that spraying the mulberry leaf enzyme solution of different fermentation periods could improve antioxidant enzyme activities (SOD, CAT, POD), but had little effect on the activity of APX. moreover, sprayed fermented for 50 d mulberry leaf enzyme solution had the most obvious effect on enhancing antioxidant enzyme activity.

Principal Component Analysis

Principal Component Analysis of the Morphology of Hydroponic Chinese Cabbage

The results of PCA showed two Principal Components (PCs) with eigenvalues higher than 1 (Fig. 4, Table 8), accounting for 34.10 and 29.22% of the total variance, respectively. PC1 was mainly related to plant width, root volume, epigeal f w, and epigeal d w; PC2 was related to plant height, leaf area, root f w, and root d w (Table 8). These results indicated that adding an appropriate concentration of mulberry leaf enzyme could significantly increase the plant width, root volume, and epigeal part fresh and dry weight of hydroponic Chinese cabbage.

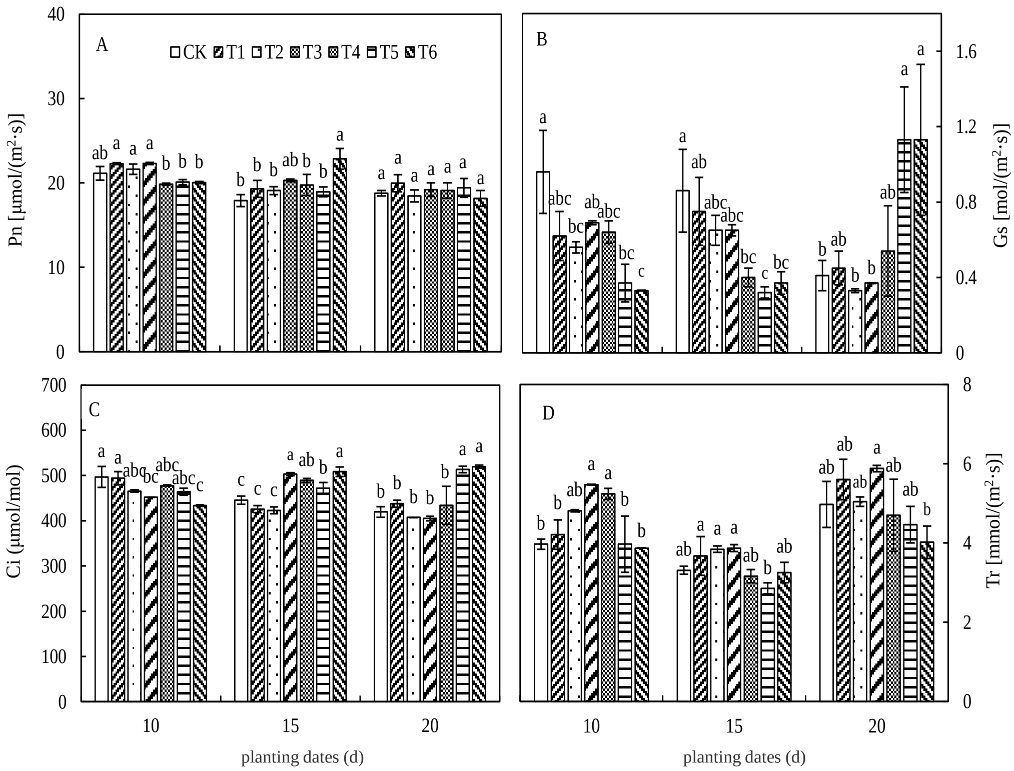

Fig. 1: Effects of spraying mulberry leaf enzyme solution on photosynthetic characteristics of hydroponic Chinese cabbage

Table 6: Effects of spraying mulberry leaf enzyme solution on the nutritional quality of hydroponic Chinese cabbage

Treatment	Soluble sugar content (mg/g)	Soluble protein (mg/g)	Vc content (mg/100g)	Nitrate nitrogen content (ug/g)	Anthocyanin content (unit/g)
CK	0.94±0.04^{c}	2.44±0.53^{a}	14.67±0.93^{b}	1706.62±70.00^{a}	1.64±0.07^{b}
T1	0.96±0.03^{ab}	2.95±0.04^{a}	15.50±0.58^{ab}	1379.30±54.23^{b}	1.70±0.02^{ab}
T2	1.07±0.03^{b}	3.39±0.20^{a}	15.83±0.67^{ab}	1353.70±43.58^{b}	1.83±0.03^{ab}
T3	1.19±0.02^{a}	3.44±0.23^{a}	18.17±0.60^{a}	1261.69±08.27^{b}	1.97±0.04^{a}
T4	0.94±0.02^{c}	2.91±0.03^{a}	15.67±1.67^{ab}	1317.13±39.74^{b}	1.77±0.16^{ab}
T5	0.91±0.03^{c}	2.74±0.29^{a}	17.83±0.73^{a}	1580.40±38.32^{a}	1.79±0.11^{ab}
T6	0.91±0.08^{c}	2.67±0.53^{a}	15.33±0.88^{ab}	1616.77±25.64^{a}	1.67±0.04^{b}

Table 7: Effects of spraying mulberry leaf enzyme solution on mineral element accumulation of hydroponic Chinese cabbage

Plant part	Treatment	Total nitrogen content (%)	Total phosphorus content (mg/g)	Potassium content (mg/g)	Calcium content (mg/g)	Magnesium content (mg/g)	Iron content (μg/g)	Zinc content (μg/g)
Shoot	CK	5.93±0.53^{a}	2.46±0.06^{b}	4.94±0.09^{cd}	20.43±0.53^{d}	5.70±0.017^{c}	30.13±1.27^{b}	24.50±2.29^{c}
	T1	6.39±0.73^{a}	2.79±0.12^{a}	5.16±0.03^{bc}	20.94±0.29^{d}	6.75±0.095^{b}	17.53±0.53^{c}	32.50±3.69^{d}
	T2	5.91±0.37^{a}	2.38±0.03^{b}	5.35±0.15^{ab}	27.10±0.09^{c}	7.93±0.003^{b}	39.00±0.58^{a}	29.67±1.45^{abc}
	T3	5.99±0.12^{a}	2.52±0.14^{ab}	5.36±0.02^{ab}	31.99±0.01^{a}	8.59±0.535^{a}	40.50±3.28^{a}	27.00±1.26^{bc}
	T4	6.23±0.19^{a}	2.53±0.09^{ab}	5.28±0.05^{ab}	30.38±0.18^{b}	8.16±0.021^{a}	10.78±1.02^{d}	34.00±0.29^{a}
	T5	6.07±0.35^{a}	2.58±0.04^{ab}	4.81±0.07^{d}	21.52±0.42^{d}	6.95±0.400^{b}	7.00±0.95^{d}	31.50±1.15^{ab}
	T6	5.96±0.13^{a}	2.49±0.09^{ab}	5.45±0.02^{a}	18.32±0.50^{e}	5.78±0.052^{c}	6.85±0.48^{d}	32.75±0.43^{ab}
Root	CK	3.16±0.17^{d}	11.62±0.62^{a}	1.17±0.06^{e}	44.73±0.12^{a}	4.65±0.15^{a}	24.90±2.03^{a}	79.67±6.09^{a}
	T1	4.26±0.12^{a}	11.27±0.40^{ab}	1.76±0.04^{b}	32.76±0.37^{e}	3.10±0.10^{c}	22.85±1.44^{a}	67.83±3.18^{b}
	T2	4.05±0.21^{ab}	9.20±0.25^{c}	2.22±0.02^{a}	32.48±0.28^{e}	3.82±0.22^{b}	24.17±1.03^{a}	83.50±3.04^{a}
	T3	3.88±0.29^{abc}	9.74±0.87^{bc}	1.37±0.06^{d}	32.46±0.09^{e}	2.42±0.18^{d}	26.23±0.87^{a}	49.83±0.73^{c}
	T4	3.13±0.18^{d}	12.13±0.80^{a}	1.35±0.07^{d}	36.06±0.52^{d}	4.30±0.15^{a}	6.35±0.33^{b}	65.17±2.73^{b}
	T5	3.40±0.14^{cd}	11.29±0.26^{ab}	1.52±0.04^{c}	41.64±0.38^{b}	2.63±0.10^{d}	25.30±3.20^{a}	47.67±4.09^{c}
	T6	3.52±0.07^{bcd}	10.87±0.17^{abc}	1.65±0.01^{bc}	38.33±0.65^{c}	2.53±0.10^{d}	20.92±1.20^{a}	41.83±1.33^{c}

Table 8: Principal component analysis of the morphology of hydroponic Chinese cabbage

Variables	PC1 (34.10%)	PC2 (29.22%)
Plant height	0.5255	-0.8432
Plant width	0.9184	0.1654
Leaf number	0.3796	-0.6264
Maximum root length	0.2496	0.5347
Root volume	0.8676	0.4128
Leaf area	0.7396	-0.8419
Epigeal part fresh weight	0.9836	0.2378
Root fresh weight	0.2231	-0.8535
Epigeal part dry weight	1.0305	0.2670
Root dry weight	-0.3449	-0.9899

Values in bold within the same factor indicate the variable with the largest correlation, as the following table

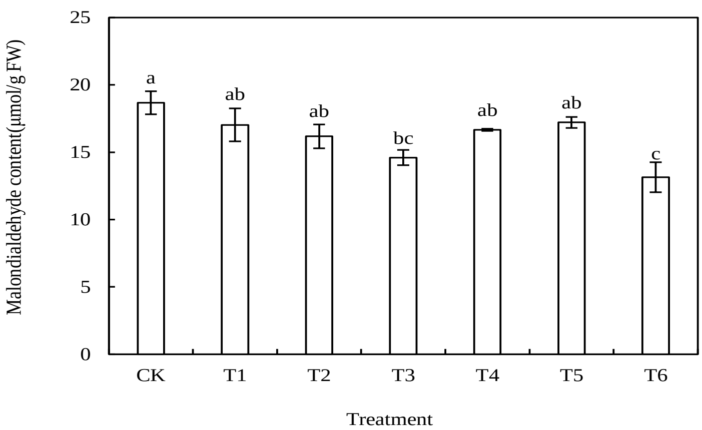

Fig. 2: Effects of spraying mulberry leaf enzyme solution on the content of malondialdehyde of hydroponic Chinese cabbage. Note: CK-Spray distilled water; In T1-T6 treatment, the fermentation days of spraying mulberry leaf enzyme solution were 20, 35,50,65,80, and 95. Values followed by different small letters indicate significant differences among treatments in the same experiment (P<0.05). as shown in the figures below

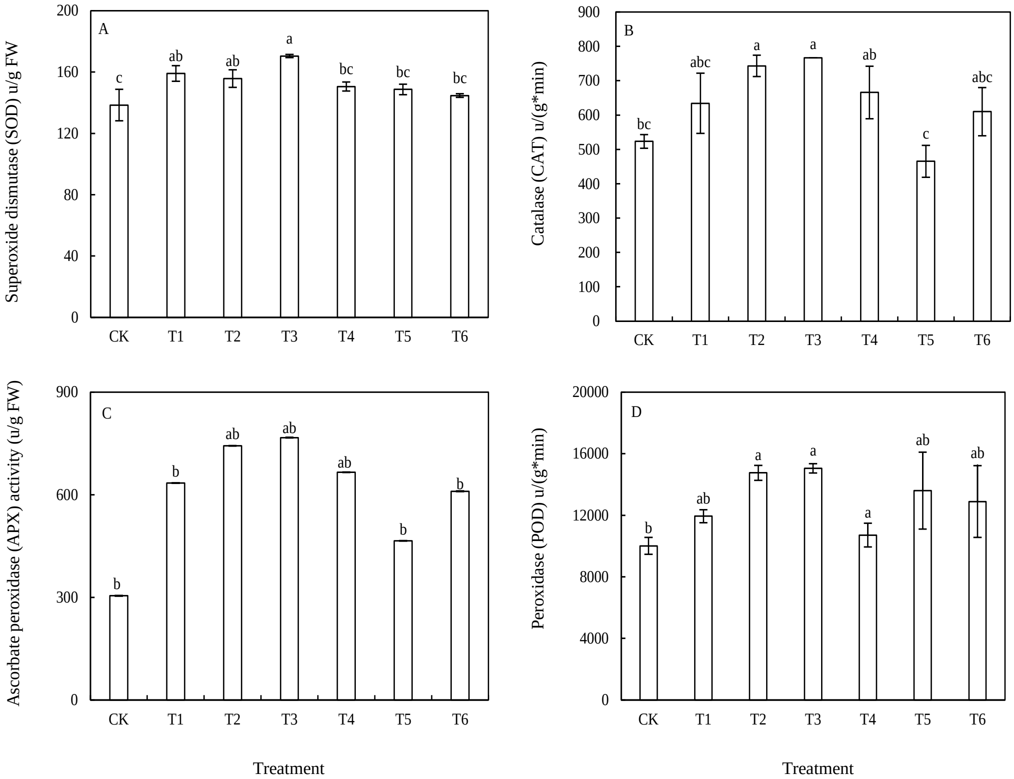

Fig. 3: Effects of spraying mulberry leaf enzyme solution on the antioxidant enzyme system of hydroponic Chinese cabbage

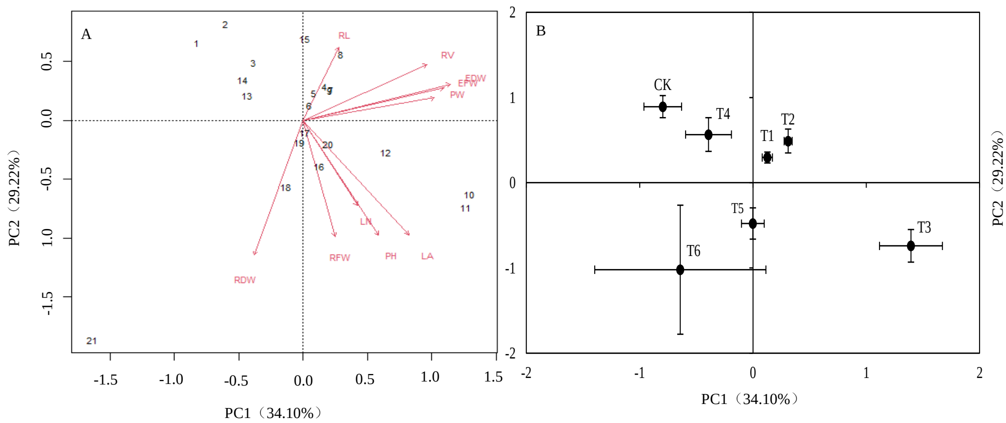

Fig. 4: A, B PCA of the relationship of the morphology under different treatments, represents the growth rate of morphological parameters of Chinese cabbage. PH, plant height; PW, plant width; LN, leaf number; RL, maximum root length; RV, root volume; LA, leaf area; EFW, epigeal part fresh weight; RFW, root fresh weight; EDW, epigeal part dry weight; RDW, root dry weight. The direction and length of the red arrows indicate the correlation and its length, respectively. Black numbers represent different treatments: 1-3 correspond to CK, 4-6 correspond to T1, 7-9 correspond to T2, 10-12 correspond to T3, 13-15 correspond to T4, 16-18 correspond to T5, 19-21 correspond to T6

Principal Component Analysis of Quality of Hydroponic Chinese Cabbage

The results of PCA showed two Principal Components (PCs) with eigenvalues higher than 1 (Fig. 5, Table 9), accounting for 52.07 and 13.74% of the total variance, respectively, this indicated that the initial 8 variables could be expressed as a linear combination of two PCs, explaining 65.81% of the total variance. PC1 was mainly related to chlorophyll a, chlorophyll b, carotenoid, chlorophyll, and anthocyanin content; PC2 was related to soluble protein content (Table 9).

Table 9: Principal component analysis of the quality of hydroponic Chinese cabbage

Variables	PC1 (52.07%)	PC2 (13.74%)
Soluble sugar content	0.7717	-0.63266
Soluble protein content	0.6915	-0.82714
Vc content	0.5285	0.10580
Chlorophyll a content	1.1003	0.49526
Chlorophyll b content	0.9327	-0.09262
Carotenoid content	0.9700	0.35266
Chlorophyll content	1.1580	0.41724
Anthocyanin content	0.9332	-0.29955

Table 10: Principal component analysis of mineral elements in roots and enzyme activities in leaves of Chinese cabbage

Variables	PC1 (48.06%)	PC2 (15.81%)
K	0.7373	0.6178
Ca	1.0714	-0.3047
Mg	1.0318	-0.3371
Fe	1.0374	-0.2402
SOD	0.9142	-0.2408
POD	0.6803	0.4095
CAT	0.9667	0.2972
APX	0.1198	1.0198

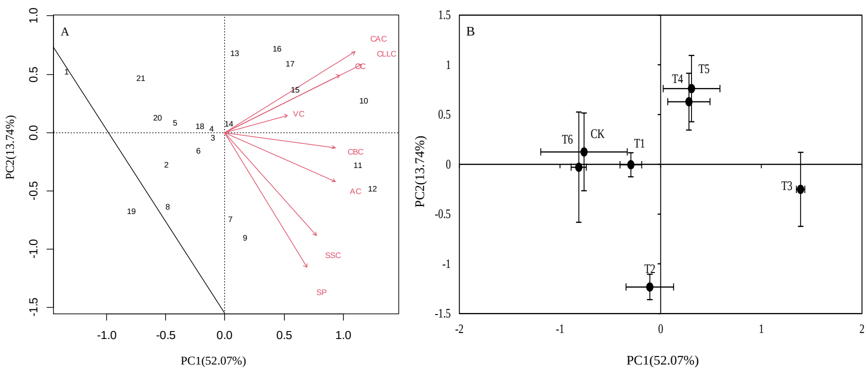

Fig. 5: A, B PCA of the relationship of the quality under different treatments, represents the growth rate of quality parameters of Chinese cabbage. SSC, soluble sugar content; SP, soluble protein content; VC, Vc content; CAC, chlorophyll a content; CBC, chlorophyll b content; CC, carotenoid content; CLLC, chlorophyll content; AC, anthocyanin content; NN, nitrate nitrogen content. The direction and length of the red arrows indicate the correlation and its length, respectively. Black numbers represent different treatments: 1-3 correspond to CK, 4-6 correspond to T1, 7-9 correspond to T2, 10-12 correspond to T3, 13-15 correspond to T4, 16-18 correspond to T5, 19-21 correspond to T6

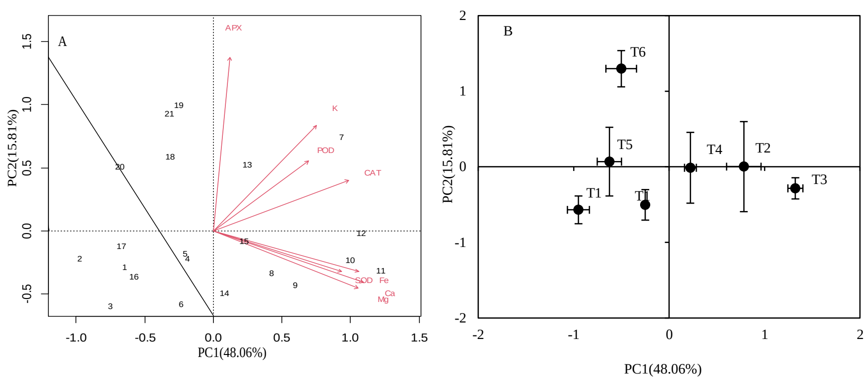

Fig. 6: A, B PCA of the relationship of the nutrient element content, the activity of enzymes under different treatments, represents the nutrient element content, activity of enzymes parameters of Chinese cabbage. K; Mg; Fe; Ca; Zn; SOD, superoxide dismutase; POD, peroxidase; CAT, catalase; MDA, malondialdehyde content; APX, ascorbate peroxidase. The direction and length of the red arrows indicate the correlation and its length, respectively. Black numbers represent different treatments: 1-3 correspond to CK, 4-6 correspond to T1, 7-9 correspond to T2, 10-12 correspond to T3, 13-15 correspond to T4, 16-18 correspond to T5, 19-21 correspond to T6

The Mineral Elements and Enzyme Activity of Chinese Cabbage Components Analysis

The results of PCA showed two Principal Components (PCs) with eigenvalues higher than 1 (Fig. 6, Table 10), accounting for 48.06 and 15.81% of the total variance, respectively, this indicated that the initial 8 variables could be expressed as a linear combination of two PCs, explaining 63.87% of the total variance. PC1 was mainly related to Ca, Mg, Fe, SOD, and CAT; PC2 was related to APX (Table 10).

Discussion

Effects of Spraying Mulberry Leaf Enzyme Solution on Growth of Hydroponic Chinese Cabbage

Mulberry leaf not only contains various nutrients, such as mineral elements, proteins, amino acids, and fatty acids but also contains a variety of trace bioactive compounds, such as phenols, alkaloids, flavonoids, and polysaccharides (Yang *et al*., 2021). Relevant studies have shown that spraying enzyme solution on leaves can promote the growth of leafy vegetables and improve yield and quality. In this experiment, sprayed mulberry leaf enzyme solution of different periods in hydroponic Chinese cabbage significantly increased plant height, plant width, leaf area, maximum root length, root volume, dry and fresh weight of aboveground and underground parts and effectively promoted the vegetative growth of hydroponic Chinese cabbage, among which T3 had the best promotion effect (Table 3). Ibrahim (2019) studies showed that foliar application of liquid organic fertilizer gave highly significant values for fenugreek plants' height and weight of seeds, this result is consistent with the results of this experiment, so sprayed mulberry leaf enzyme solution can significantly improve the nutrient growth of hydroponic Chinese cabbage. However, the mulberry leaf enzyme contains a variety of amino acids, organic substances, and other components and the effect of spraying enzymes in different periods on the growth of hydroponic Chinese cabbage is significant and the key factors that promote the growth need to be further studied.

Effects of Spraying Mulberry Leaf Enzyme Solution on Nutritional Quality of Hydroponic Chinese Cabbage

vitamin C is known to play various roles in nutrition and human health as an antioxidant (Navarre *et al*., 2010), vitamin C deficiency will lead to the occurrence of a variety of diseases and it cannot be synthesized by the human body, so fruits and vegetables are its major sources (Cho *et al*., 2013). In addition, the highest level of nitrate is supplied to humans by green leafy vegetables (Prasad and Chetty, 2008), they also contain nitrate and nitrite, which are harmful to humans when taken in excess (Mazahar *et al*., 2015), therefore, nitrate content is also one of the important nutritional indicators for evaluating vegetables. Sugar is the main energy substance in plants and can release energy to meet survival needs through its biological oxidation or be stored as an energy reserve (Tang *et al*., 2021). Soluble proteins in plants are mostly enzymes involved in various metabolism and their content is an important indicator to understand the total metabolism of plants (Tang *et al*., 2021). In this experiment, soluble sugar, Vc, and anthocyanin contents in hydroponic Chinese cabbage were significantly increased by sprayed mulberry leaf enzyme solution. Compared with CK, they were increased by 26.60, 23.86 and 20.12%, respectively, they are consistent with Huang *et al*. (2018) research results that sprayed plant enzymes on amaranth effectively improved quality indexes of amaranth, such as Vc, soluble sugar content. In addition, high nitrate levels in vegetables constitute a health hazard, such as cancers and blue baby syndrome (Wang *et al*., 2009), sprayed mulberry leaf enzyme solution significantly reduced nitrate content in hydroponic Chinese cabbage, and nitrate nitrogen content of T3 reached the lowest level, which was significantly reduced by 35.26% compared with CK. The results showed that spraying mulberry leaf enzyme solution at different periods had different effects on the quality of Chinese cabbage, which was mainly related to the complex components in the mulberry leaf enzyme solution. However, this experiment simply studied the soluble sugar, protein, Vc, and other indexes of mulberry leaf enzyme solution in hydroponic Chinese cabbage, the key factor and its influence on the specific mechanism remains to be further researched.

Effects of Spraying Mulberry Leaf Enzyme Solution on Photosynthetic Pigment Content and Photosynthetic Characteristics of Hydroponic Chinese Cabbage

The leaf is the main organ for photosynthesis and chlorophyll is the basic substance for the photosynthesis of green plants, it is also an important indicator of nutritional stress, photosynthetic capacity, and growth status of plants (Cui and Zhou, 2017). Carotenoids are natural pigments and antioxidants found in fruits and vegetables such as carrot, tomato, orange, mango, yellow corn, pumpkin, and mamey (González-Peña *et al*., 2021), it plays an important role in human health. In this experiment, sprayed mulberry leaf enzyme solution significantly increased the contents of chlorophyll a, chlorophyll pigment, and carotenoid in hydroponic Chinese cabbage, among which T3 was the largest and increased by 53.90 59.52 and 22.37% compared with CK, respectively, (Table 4). Yan and Pei (2014) showed that spraying different kinds of foliar fertilizer could increase chloroplast content, pigment photosynthetic activity of wheat, reduced heat energy dissipation, increased photochemical energy conversion efficiency of leaves, and increased photosynthetic rate, which was consistent with the results of this experiment. Hydroponic Chinese cabbage showed the photosynthetic characteristics of different in different growth periods, this may be because the growth is slower with less chlorophyll synthesis, grows strong middle reaches its peak value of photosynthesis, photosynthesis decreased at a later growth stage and Chinese cabbage absorption of nutrient supply different during different periods, prophase effect is not obvious, but as the growth of plants, gradually deficient nutrient supply, the chlorophyll synthesis in Chinese cabbage leaves was affected and the photosynthetic characteristics were decreased.

Effects of Spraying Mulberry Leaf Enzyme Solution on the Content of Malondialdehyde and the Antioxidant Enzyme System of Hydroponic Chinese Cabbage

Accumulation of MDA, a product of lipid peroxidation, is a good indicator of the severity of cell injury during oxidative stress (Xin *et al*., 2007). Plants have formed a complex antioxidant enzyme system in the long-term evolution process to remove excess ROS, like Catalase (CAT), Peroxidase (POD), and Superoxide Dismutase (SOD) (Zhao *et al*., 2022). Mantzourani *et al*. (2018) demonstrated an improvement in antioxidant activity, as well as a higher phenolic content in fermented pomegranate juice. In this experiment, SOD, CAT, and POD activities in hydroponic Chinese cabbage were significantly increased by spraying mulberry leaf enzyme solution. Heilili *et al*. (2018) study showed that sprayed foliar fertilizer can significantly increase protective enzyme activities (CAT, POD, SOD) in cotton leaves, then enhanced the ability of cotton to resist adversity stress, this is consistent with the results of this experiment.

Effects of Spraying Mulberry Leaf Enzyme Solution on the Content of Mineral Elements of Hydroponic Chinese Cabbage

Mineral element contents in different types of vegetables are different and Ca, Cu, Zn, Fe and Mn contents in leaf vegetables are higher than that in fruit vegetables and root vegetables (Li *et al*., 2000). Therefore, the content of mineral elements absorbed is one of the important indicators for evaluating the quality of vegetables. In this experiment, sprayed mulberry leaf enzyme solution could significantly increase the contents of P, K, Ca in leaves and N, K in roots of Chinese cabbage. Wang *et al*. (2009) showed that enzyme bacteria biofertilizer could promote the absorption of N, P, and K nutrients in tomatoes at all growth stages and improve the nutrient content in fruit, all these are consistent with the results of this experimental study.

This experiment only discussed the effects of foliar applications of mulberry leaf enzyme solution fertilizer on growth, nutritional quality, photosynthetic characteristics, mineral elements, and antioxidant enzyme system of hydroponic Chinese cabbage, and the molecular regulation mechanism in plants needs to be further studied.

Conclusion

In the experiment, sprayed mulberry leaf enzyme solution of different periods in hydroponic Chinese cabbage, could significantly increase plant height, plant width, leaf area, dry fresh weight and promote growth, it also can significantly increase soluble sugar, Vc, and anthocyanin contents, improve nutritional quality in hydroponic Chinese cabbage, it also effectively enhanced photosynthesis, increased chlorophyll content, increased net photosynthetic rate and stomatal conductance of leaves and improved photosynthetic performance. In addition, it can effectively improve the antioxidant enzyme system (SOD, CAT, POD) and enhance the antioxidant capacity of hydroponic Chinese cabbage. Considering the morphology, quality and photosynthetic performance of Chinese cabbage in hydroponic culture, the best fermentation days of mulberry leaf enzyme solution were 50 d.

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Author’s Contributions

Li Yang: Participated in all experiments, conceived the project, designed the experiments and wrote the draft manuscript.

Fan Yuting: Conducted the experiments and analyzed the data.

Du Qingjie: Wrote the draft manuscript.

Xiao Huaijuan: Wrote the draft manuscript and assisted with the data analysis.

Wang Jiqing: Assisted with the data analysis.

Li Juanqi: Provided the facility and funding.

All authors read and approved the final version of this manuscript.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues involved.

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