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Effects of natural plant growth regulator iron chlorin on photosynthesis, yield, and quality of watermelons grown in greenhouses

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Abstract: Iron chlorin is known to affect plant growth, but its potential applications in watermelon production have rarely been explored. To better understand its effects on the growth, photosynthesis, yield, and quality of watermelon in a greenhouse setting, a series of experiments were conducted using the variety 'Sumeng 6'. At the flowering and early fruit expansion stages, the plants were sprayed with iron chlorin with mass concentrations of 0.001, 0.002, 0.004, and 0.008 μg/L (T1, T2, T3, and T4). Control plants were sprayed with water (CK). The growth index, root activity, photosynthetic pigment content, photosynthetic parameters, yield, and fruit quality of all plants were measured. The results showed: compared with CK, the T3-treated plants showed significant improvements in several aspects: the leaf contents of photosynthetic pigments, chlorophyll *a*, total chlorophyll, and carotenoid, increased by 19.51%, 14.29%, and 29.17%, respectively ($P < 0.05$); the net photosynthetic rate (*Pn*) increased by 23.60% ($P < 0.05$); and the soluble solids content, vitamin C content, and yield increased by 7.89%, 34.13%, and 16.27%, respectively ($P < 0.05$). In summary, it was found that spraying 0.004 μg/L iron chlorin on facility watermelon plants at the flowering and the early fruit expansion stages has a significant effect on the promotion of growth and development, leading to improved quality and yield. This study provides a theoretical reference and technical guide for high-quality and efficient watermelon production.

Keywords: watermelon; iron chlorin; growth; vitamin C; photosynthesis

Plant growth regulators commonly used in agricultural production include growth hormone auxin (IAA), gibberellin (GA), brassinolide (BL), ethylene (ET), salicylic acid (SA), etc. (Hanna et al. 2022). Plant growth regulators have the function of regulating plant growth and development (Svoboda et al. 2021), which can enhance plant resistance and achieve increased yield and quality (Waqas et al. 2012). Iron chlorin is a new type of plant growth regulator newly developed in recent years, which belongs to chlorophyll derivatives, is a natural plant growth regulator extracted from silkworm sand and has significant ef-

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fects such as resistance to adversity, resistance to disease, increase in yield, and promotion of root and seedling growth (Xiao et al. 2022; Zhang et al. 2022; Li et al. 2023). Studies have shown that ferric chloride can inhibit the activity of chlorophyll enzyme, delay the degradation of chlorophyll and improve the photosynthetic efficiency of plants (Xing et al. 2020a). Iron chlorin has demonstrated obvious disease resistance in many crops, such as rice, wheat, pepper, etc., and at the same time, it has a significant effect of resistance to adversity and yield increase in rice, wheat, pepper, potato, peanut, soybean, and other do-it-yourself crops, and it has obtained the national pesticide registration in December 2018 (Bai et al. 2018). Some studies have shown that 0.02% iron chlorin soluble powder has a significant promotion effect on plant height, leaf length, leaf width, and single leaf mass of tobacco, as well as increasing its yield (Xing et al. 2020a). Spraying 0.02% iron chlorin soluble powder at the break stage and full heading stage of rice could shorten the growth period of rice by 3 days and increase the yield by 9.6%. The yield increase effect was obvious, and it was safe for rice without side effects (Zhao et al. 2022). The use of iron chlorin in chili peppers (Liu et al. 2023), grapes (Xing et al. 2020b) and other crops also has the effect of promoting plant growth, increasing and stabilizing yields.

Watermelon [*Citrullus lanatus* (Thunb.) Matsum. and Nakai] is one of the most important horticultural crops in China and even the world, widely cultivated worldwide (Garcia-Lozano et al. 2020). China is the world's largest watermelon producer (Shireen et al. 2020), according to the Food and Agriculture Organization of the United Nations, in 2019, China's watermelon planting area reached 1 471 580 hm^2, accounting for about 1/2 of the world's total cultivated area of watermelon, and the production amounted to 63.241 million t, accounting for more than 1/2 of the world's total watermelon production (Chen et al. 2023). Therefore, watermelon industry has become an important industry in China to improve farmers' income and promote agricultural economic development (Guo et al. 2022). Watermelon flesh is sweet, rich in mineral salts and a variety of vitamins (Carolina et al. 2023). Facility cultivation is the main efficient development mode of modern melon and fruit vegetables, and the cultivation area is increasing rapidly year by year (Zhang et al. 2023a). At present, iron chlorin has been studied in rice, soybean, grapes, oilseed rape and other crops, but there is little research on the application of watermelon in facilities.

Watermelon is an important global fruit and an important melon crop in China, and the research on the application of plant growth regulators on watermelon is of great significance to the green, high-quality, high-efficiency and high-quality production of watermelon. Therefore, this experiment takes watermelon as the test material, to study the effect of exogenous application of iron chlorin on the photosynthesis and growth of watermelon, and to explore the cultivation technology suitable for high yield and high efficiency of watermelon in facilities, with a view to providing theoretical support and technical guidance for high yield and high efficiency of watermelon.

MATERIAL AND METHODS

Experimental material. Test variety: 'Sumeng 6' watermelon, seeds purchased from Jin Yangpu (Beijing) Agricultural Science and Technology Co., Ltd. The variety has excellent characteristics such as stable growth, good early maturity, high sugar content, good taste, good fruit setting and good resistance.

Test reagent: 0.02% iron chlorin soluble powder, purchased from Nanjing Biotek Biological Engineering Co., Ltd.

Experimental design. This experiment was carried out from March 2022 to June 2022 in a continuous greenhouse (40 m in length and 16 m in span) at the National protected Agriculture R&D Center, Institute of Protected Agriculture, Academy of Agricultural Planning and Engineering, Ministry of Agriculture and Rural Affairs (116.44'E, 39.23'N). Watermelon was grown in elevated coco coir strips (specifications of 1 m length, 20 cm width, and 10 cm height) with row spacing of 160 cm and plant spacing of 25 cm. Fertilization adopts intelligent management of water and fertilizer integrated machine, and fertilizer adopts water-soluble fertilizer for watermelon and melon, which is provided by Shanghai Meini Ecological Agriculture Co., Ltd. The daily irrigation amount of watermelon after planting was 50–100 mL/plant, and the daily irrigation amount during the expansion period was 400–500 mL/plant. At the seedling stage of watermelon, the daytime temperature was 25–28 °C, the nighttime temperature was ≥ 15 °C, and the relative humidity was 50–65%. During the watermelon vine extension period, the daytime temperature was 27–32 °C, the nighttime temperature was ≥ 16 °C, and the relative humidity was 60–70%. During the fruit setting period,

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the daytime temperature was 25–32, the nighttime temperature was ≥ 18 °C, and the relative humidity was 60–70%. During the harvest period, the daytime temperature was 28–32 °C, the nighttime temperature was 15–18 °C, and the relative humidity was 60–70%. Seeds were sown on February 23, 2022, planted on March 31, 2022, and harvested on May 31, 2022.

The experiment was conducted by foliar spraying, in which iron chlorin soluble powder with 0.02% mass fraction was prepared into four horizontal concentration solutions with water, i.e., mass concentrations of 0.001, 0.002, 0.004, 0.008 μg/L (set as T1, T2, T3, T4, respectively), and spraying with water was used as the control (set as CK). Two watermelon plants were set aside as a buffer zone between each treatment to ensure that the treatments did not interfere with each other. The whole leaves of watermelon plants were sprayed at the flowering stage (April 21, 2022) and the early fruit expansion stage (May 7, 2022), and a total of two sprays were applied at a rate of about 180 mL/plant to ensure that the liquid was evenly distributed on the leaf surface and did not drip down, and a fruit was left in each plant. Leaf SPAD value, photosynthetic pigment content and photosynthetic parameters were determined after watermelon set (May 15, 2022), and growth indexes, root vigour, yield and quality were determined at maturity (May 31, 2022).

Measurement indicators and methods.

Determination of growth indexes and root vigour. Each treatment was repeated three times, and five watermelon plants with the same growth were selected for each repetition. A straightedge was used to measure the height of the plant, and vernier calipers were used to measure the thickness of the stem at the base, while the part of the stem above the base was cut, and the aboveground part was weighed by an electronic counting scale to determine the fresh mass; the samples were placed in a electric blast drying oven (model: 101-1A, Tianjin Taiste Instrument Co., Ltd., Tianjin, China) oven at 105 °C for 15 minutes, dried at a constant temperature of 75 °C until the mass was constant, and then the dry mass was weighed. Its dry mass; each treatment selected three watermelon plants with the same growth, using the TTC method to determine root vigour (Hao et al. 2019).

Determination of photosynthetic pigment content. Three watermelon plants with the same growth were selected for each treatment, and the photosynthetic pigment content of leaves was determined by the leaching method. The fresh leaves were cut into small pieces of about 0.2 cm and mixed evenly. Weigh 0.20 g and put it into the test tube. Add 0.5 mL pure acetone and 15 mL 80% acetone to the test tube, cover the bottle stopper, and extract overnight in the dark at room temperature. Shake 3–4 times during the period. The next day, the test tube was taken out. When the leaf tissue was completely whitened, it was diluted with 80% acetone to 25 mL. After filtration, it was determined by spectrophotometer with 80% acetone as blank control (Wang et al. 2023).

Determination of photosynthetic parameters. The LI-6400 photosynthesis meter (USA) was used for the determination. Three watermelon plants with the same growth were selected for each treatment, and the net photosynthetic rate (*Pn*), transpiration rate (*Tr*), intercellular CO_2 concentration (*Ci*) and stomatal conductance (*Gs*) of watermelon plant leaves (the fourth functional leaf counted down from the growth point) were measured from 9:00 a.m. to 11:00 a.m. on a sunny day. The photosynthetic photon flux density (PPFD) was set at 500 μmol/m/s, the CO_2 concentration was 500 μmol/mol, the leaf chamber temperature was (25 ± 1) °C, and the relative humidity was 60–70%.

Yield and quality measurement. During the ripening period of watermelon, each treatment was replicated three times, and five commercial fruits were selected in each replication for weighing and counting the yield; three fruits were selected for each treatment, and the tetrad method was adopted; after peeling the watermelon, the fruits were churned with a tissue churning machine and mixed homogeneously, and the quality indexes of the watermelon were determined, and the soluble solids content was determined by using a digital refractometer, and the vitamin C content was determined by the titration method according to 2,6-dichloroindophenol (Feszterová et al. 2023).

Data processing. Microsoft Excel 2010 was used to process the data; SPSS 17.0 software was used to analyze the data by one-way ANOVA, and the Duncan test was applied for multiple comparisons of the significance of the differences ($P < 0.05$ indicates significant differences between treatments).

RESULTS

Effects of iron chlorin on growth index and root activity of watermelon plants. As shown in Table 1,

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Table 1. Effects of iron chlorin on growth index and root activity of watermelon plants

Treatment	Plant height (cm)	Stem thickness (mm)	Aboveground fresh weight (g)	Aboveground dry weight (g)	Root activity (μg/g/h)
CK	323.67 ± 12.10[a]	5.62 ± 0.09[c]	295.33 ± 23.49[c]	32.83 ± 3.21[b]	15.54 ± 0.11[d]
T1	325.33 ± 17.62[a]	6.32 ± 0.33[b]	351.83 ± 15.83[a]	40.17 ± 2.02[a]	16.61 ± 1.10[d]
T2	324.00 ± 14.11[a]	6.13 ± 0.24[b]	315.17 ± 11.75[bc]	37.67 ± 3.79[ab]	28.99 ± 1.42[b]
T3	321.00 ± 10.54[a]	6.91 ± 0.22[a]	362.67 ± 20.64[a]	43.33 ± 3.88[a]	31.59 ± 2.01[a]
T4	336.33 ± 11.59[a]	6.46 ± 0.22[b]	343.67 ± 10.49[ab]	38.33 ± 1.26[ab]	24.56 ± 0.75[c]

CK – water; T1, T2, T3, and T4 represent iron chlorins with mass concentrations of 0.001, 0.002, 0.004, and 0.008 μg/L, respectively; [a–d]significant difference among treatments at 0.05 level ($P < 0.05$) by Duncan's test

spraying the appropriate concentration of iron chlorin significantly increased the stem thick, aboveground fresh weight and aboveground dry weight of watermelon plants, but there was no significant difference between the treatments in terms of plant height. Compared with CK, the stem diameter of T1, T2, T3 and T4 treatments increased significantly by 12.46%, 9.07%, 22.95% and 14.95%, respectively ($P < 0.05$), with T3 treatment having the thickest stem; Meanwhile, the aboveground fresh weight of T1, T3 and T4 treatments were significantly increased by 19.13%, 22.80% and 16.37% ($P < 0.05$), respectively, compared to CK, with T3 treatment having the best effect; the aboveground dry weight of T1 and T3 treatments was significantly increased by 22.36% and 31.98%, respectively, compared to CK ($P < 0.05$), and there was no significant differences. Root activity is an important index reflecting the physiological processes of plant root growth, absorption and metabolism (Zhang et al. 2023b). Compared with CK, the root activity of T2, T3 and T4 treatments were significantly increased by 86.55%, 103.28% and 58.04% ($P < 0.05$), respectively, with the greatest increase in T3. In summary, T3 treatment increased stem thickness, increased the plant biomass, and promoted the root growth of watermelon most significantly.

Effect of iron chlorin on photosynthetic pigments in watermelon leaves. Leaf photosynthetic pigment content is an important parameter to measure the photosynthetic capacity and growth of plants (Li et al. 2020). As shown in Table 2, spraying a certain concentration of iron chlorin significantly increased the photosynthetic pigment content of watermelon leaves. The chlorophyll content of T3 treatment significantly increased by 19.51% compared with CK ($P < 0.05$); the total chlorophyll content of T3 treatment significantly increased by 14.29% compared with CK ($P < 0.05$); at the same time, compared with CK, the carotenoid content of T2 and T3 treatments significantly increased by 20.83% and 29.17%, respectively ($P < 0.05$). In summary, T3 treatment significantly increased the photosynthetic pigment content of watermelon and promoted the absorption and transfer of light energy, thus improving the photosynthetic efficiency of the plant.

Effects of iron chlorin on photosynthetic parameters of watermelon leaves. Photosynthetic parameters are important indicators for evaluating the photosynthetic capacity of plants and are closely related to the growth and development of plants. As can be seen from Table 3, spraying the appropriate concentration of iron chlorin can improve the photosynthetic capacity of plants to a certain extent, in which

Table 2. Effect of iron chlorin on photosynthetic pigments in watermelon leaves

Treatment	Chlorophyll *a* content (mg/g)	Chlorophyll *b* content (mg/g)	Total chlorophyll content (mg/g)	Carotenoid content (mg/g)
CK	1.23 ± 0.02[b]	0.45 ± 0.03[a]	1.68 ± 0.03[b]	0.24 ± 0.02[b]
T1	1.26 ± 0.13[b]	0.46 ± 0.04[a]	1.72 ± 0.17[b]	0.26 ± 0.01[b]
T2	1.30 ± 0.07[b]	0.42 ± 0.02[a]	1.72 ± 0.06[b]	0.29 ± 0.01[a]
T3	1.47 ± 0.08[a]	0.45 ± 0.05[a]	1.92 ± 0.03[a]	0.31 ± 0.01[a]
T4	1.20 ± 0.07[b]	0.41 ± 0.03[a]	1.61 ± 0.04[b]	0.25 ± 0.01[b]

CK – water; T1, T2, T3, and T4 represent iron chlorins with mass concentrations of 0.001, 0.002, 0.004, and 0.008 μg/L, respectively; [a,b]significant difference among treatments at 0.05 level ($P < 0.05$) by Duncan's test

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Table 3. Effects of iron chlorin on photosynthetic parameters of watermelon leaves

Treatment	Net photosynthetic rate (µmol/m/s)	Transpiration rate (mmolm/s)	Intercellular CO_2 concentration (µmol/mol)	Transpiration rate (mol/m/s)
CK	9.28 ± 0.18^{d}	4.40 ± 0.11^{d}	206.92 ± 3.46^{a}	0.24 ± 0.01^{c}
T1	10.09 ± 0.28^{bc}	4.66 ± 0.18^{d}	198.16 ± 8.74^{ab}	0.28 ± 0.00^{b}
T2	10.28 ± 0.35^{b}	5.39 ± 0.10^{c}	195.59 ± 4.81^{b}	0.30 ± 0.02^{b}
T3	11.47 ± 0.36^{a}	6.63 ± 0.39^{a}	195.40 ± 2.03^{b}	0.36 ± 0.03^{a}
T4	9.75 ± 0.09^{cd}	6.05 ± 0.06^{b}	198.57 ± 3.38^{ab}	0.30 ± 0.02^{b}

CK – water; T1, T2, T3, and T4 represent iron chlorins with mass concentrations of 0.001, 0.002, 0.004, and 0.008 µg/L, respectively; $^{a–d}$significant difference among treatments at 0.05 level ($P < 0.05$) by Duncan's test

the net photosynthetic rate, transpiration rate and stomatal conductance showed a trend of increasing and then decreasing with the increase of the treatment concentration, and the intercellular CO_2 concentration showed a trend of decreasing and then increasing. Compared with CK, the net photosynthetic rate of T1, T2 and T3 treatments significantly increased by 8.73%, 10.78% and 23.60%, respectively ($P < 0.05$), with T3 treatment having the highest net photosynthetic rate; the transpiration rate of T3 treatment was significantly higher than that of the other treatments, with a significant increase of 50.68% compared with that of CK ($P < 0.05$); moreover, the intercellular CO_2 concentration was significantly reduced by 5.57% compared with CK ($P < 0.05$); stomatal conductance was significantly increased in T1, T2, T3 and T4 treatments compared with CK by 16.67%, 25.00%, 50.00% and 25.00%, respectively ($P < 0.05$). In summary, T3 treatment increased the net photosynthetic rate, transpiration rate, stomatal conductance and reduced the intercellular CO_2 concentration of watermelon most significantly.

Effects of iron chlorin on fruit size, flavor quality and yield of watermelon. Fruit size is an important measure of appearance quality. As can be seen from Table 4, spraying the appropriate concentration of iron chlorin could promote fruit growth, and the longitudinal diameter of fruit in T3 treatment significantly increased by 11.16% ($P < 0.05$) compared with CK; the transverse diameter of fruit in T3 and T4 treatments significantly increased by 10.09% and 5.26% ($P < 0.05$), respectively, compared with CK, and the increase was the greatest in T3 treatment. Fruit type index is the ratio of longitudinal and transverse fruit diameter (Qiao et al. 2017). 'Sumeng 6' belongs to the varieties with nearly round fruits, and there was no significant difference in fruit shape index between CK and treatments.

Fruit flavor quality is an important index to measure the quality of the fruit, and the level of yield can directly determine the amount of benefits. Spraying the appropriate concentration of iron chlorin had a significant effect on the flavor quality and yield of watermelon, and the soluble solids content of T3 treatment was significantly increased by 7.89% compared with CK ($P < 0.05$); the vitamin C content of T2, T3, and T4 treatments was significantly increased by 29.61%, 34.13%, and 20.48%, respectively, compared with CK ($P < 0.05$); The yield of T3-treated plots was significantly increased by 16.27% ($P < 0.05$) compared with CK. In conclusion, spraying the appropriate concentration of iron

Table 4. Effects of iron chlorin on fruit size, flavor quality and yield of watermelon

Treatment	Longitudinal diameter of fruit (cm)	Transverse diameter of fruit (cm)	Fruit shape index	Soluble solids content (%)	Vitamin C (mg/100/g)	Plot yield (g)
CK	14.87 ± 0.42^{b}	13.87 ± 0.50^{c}	1.07 ± 0.02^{a}	11.40 ± 0.35^{b}	11.28 ± 0.89^{d}	6 965.33 ± 655.28^{b}
T1	15.37 ± 0.85^{b}	14.10 ± 0.26^{bc}	1.09 ± 0.04^{a}	11.50 ± 0.35^{b}	12.56 ± 0.44^{cd}	7 271.00 ± 521.76^{ab}
T2	15.70 ± 0.69^{ab}	14.20 ± 0.46^{bc}	1.11 ± 0.02^{a}	11.63 ± 0.12^{ab}	14.62 ± 0.77^{ab}	7 083.67 ± 658.61^{ab}
T3	16.53 ± 0.15^{a}	15.27 ± 0.31^{a}	1.08 ± 0.03^{a}	12.30 ± 0.30^{a}	15.13 ± 0.89^{a}	8 098.67 ± 430.06^{a}
T4	15.90 ± 0.36^{ab}	14.60 ± 0.17^{b}	1.09 ± 0.02^{a}	11.27 ± 0.60^{b}	13.59 ± 0.89^{bc}	7 900.17 ± 554.28^{ab}

CK – water; T1, T2, T3, and T4 represent iron chlorins with mass concentrations of 0.001, 0.002, 0.004, and 0.008 µg/L, respectively; $^{a–d}$significant difference among treatments at 0.05 level（$P < 0.05$）by Duncan's test

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chlorin can significantly promote fruit growth, enhance the flavor quality of watermelon and increase the yield, and the T3 treatment was more effective.

DISCUSSION

Plant leaves are important organs for photosynthesis, in which photosynthetic pigments are the material basis for photosynthesis (Kreslavski et al. 2018), and photosynthetic parameters are also an important parameter for evaluating the photosynthetic capacity of plants. The results of this experiment showed that spraying the whole watermelon plant with iron chlorin at a concentration of 0.004 μg/L significantly increased the content of photosynthetic pigments and photosynthetic parameters of leaves to a certain extent, thus increasing the photosynthetic rate of the plant and promoting the growth and development of the plant. The enhancement of photosynthetic rate may be attributed to the fact that iron chlorin delays chlorophyll degradation and increases chlorophyll content by inhibiting chlorophyllase activity, thereby increasing the maximum photochemical efficiency (Fv/Fm) of photosystem II (PS II) and promoting photosynthesis in plants (Xiao et al. 2022; Zhang et al. 2022). Plant root system is an active absorption organ and synthesis organ, the more developed the root system is, the stronger the absorption capacity is, and the more vigorous its vitality is (Tomob et al. 2023). This study showed that spraying the appropriate concentration of iron chlorin can significantly improve the plant root vigour and promote root growth, and the increase in root vigor may be due to the fact that the spraying of iron chlorin twice during the pre-growth period of the plant played a role in increasing the biomass of the plant, delaying the degradation of chlorophyll, and to a certain extent increasing the photosynthesis of the plant to promote the transport of assimilates, and the aboveground can provide photosynthetic products for the root system to promote the root system's The above ground can provide photosynthetic products for the root system, and promote the growth expansion and function of the root system. In addition, iron chlorin can, to a certain extent, promote the production of NO in root cells and reduce the activity of indoleacetic acid oxidase, thus achieving the role of promoting the growth of plant roots (Xiao et al. 2022; Zhang et al. 2022).

Fruit appearance quality, inner quality and yield is an important index to measure the superiority and efficiency of the fruit. There have been studies showing that the transverse and longitudinal diameters of fruits after 0.02% iron chlorin treatment of grapes were higher than that of the control treatment, and spraying appropriate concentration of iron chlorin can significantly increase the soluble solids content of grapes (Xing et al. 2020b), which was consistent with the findings of this experiment. Spraying 0.02% iron chlorin soluble powder 5000 times at the sixth true leaf stage, the third day after transplanting, the initial flowering stage and the fruit expansion stage can effectively increase the yield. The yield of fresh pepper per $667m^2$ increased by 19.74%, and the yield of dry pepper increased by 47.01% (Liu et al. 2023); Previous studies have shown that ICE6 showed obvious promoting effects on tobacco plant height, leaf length, leaf width and single leaf quality. After application, the tobacco production raised significantly, increased by 9.34% and 21.35% per 666.7 m^2, respectively (Lyu et al. 2023). This is consistent with the results of this study.

The increase in fruit quality and yield may be due to the fact that iron chlorin improves the photosynthetic capacity and root vigour of the plant, which promotes the transport, absorption and accumulation of substances in the crop to a certain extent, and thus serves to improve the quality and yield of the crop, and the mechanism of which remains to be further studied.

CONCLUSION

Spraying 0.004 μg/L iron chlorin on watermelon plants at the flowering and early fruit expansion stages can effectively promote the growth of watermelon plants, significantly increase the content of photosynthetic pigments and photosynthetic parameters of plants, enhance the photosynthetic capacity of plants, and promote the growth of plant roots, as well as have the effect of improving the quality of fruit and increasing the yield.

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