

Full Length Research Paper

Study on the optimum nutrient solution concentration for the growth of Mint (*Mentha* genus)

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Mint (*Mentha* genus) is an herbaceous herb that is fragrant useful for centuries with spices and medicinal herbs. Study on optimum nutrient solution sufficient to meet the plants' demands regarding soilless culture of mint would be helpful for its application in plant factory, but was less reported. In the current study, 0.5 fold (x), 0.75x, 1.0x and 1.25x concentrations of Yamazaki formula nutrient solution were applied to culture the mint seedlings using river sand as the substrate. The results showed that 0.75x concentration of nutrient solution was optimum for mint seedlings across 15 to 36 days treatments, which dramatically promoted the growth rates, enhanced the content of chlorophyll, and improved yield as well as quality, followed by other desirable concentrations, ranking as 0.5x > 1.0x > 1.25x times, on growth of mint were distinct. Therefore, we concluded that the optimum concentration for mint seedling growth was 0.75x concentration, and highlighted the importance of using Yamazaki formula nutrient solution for mint seedlings growth, which potentially guides soilless cultivation of mint.

Key words: Mint, Japanese raw materials, nutrition, hydroponics.

INTRODUCTION

Mentha arvensis (Mint) is well known as mint, wild mint, lotus leaf, lotus tea, sunrise grass and nightfall (He et al., 1992). Mint belongs to the genus *Mentha* of Labiatae and it is a perennial herb. The stems and leaves of mint are edible, cool in nature and spicy in taste. It has the medicinal functions of dispersing wind-heat and relieving vertax one's nerves (Lal, 2013). Mint is mainly used to treat the symptoms of cold, fever, headache, and sore throat (Zheng, 1988). Stem and leaf of mint are also used as raw materials for extracting mint oil, menthol, menthol and other spices, and fresh mint can be directly eaten, and can be used as seasoning agent, spice, wine and tea (Wu, 2015).

In China, extensive cultivations were applied for mint,

such as in Jiangsu, Henan, Anhui, and Jiangxi (He et al., 1992). As early as 2000 years ago, the ancients of Chinese started to collect mint for edible or medicinal purposes, and many varieties were bred in China (Fang and Gao, 2005; Wang et al., 2003). Since the 1960s, chemical constituents, pharmacological effects and cultivation techniques were studied preliminarily using mint (Rao et al., 2000; Naeem et al., 2014; Baudhd and Singh, 2012; Guo et al., 2016). Among them, the volatile oil was extensively studied, and some achievements were made in terms of industrial products. In recent years, mint was widely used in cosmetics, food, medicine and spices industries, making it more and more valuable and popular (Xu, 2007).

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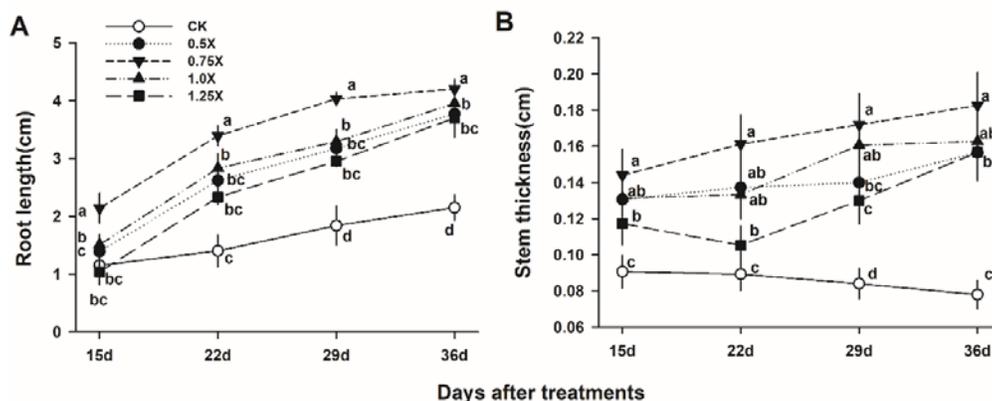


Figure 1. Effects of nutrient solutions concentrations on root and stem morphology of *Mentha* Mint. A-B, represent root length and stem thickness. Different alphabetic letters in the values of different concentrations for each time point of treatment indicate significant difference at $P < 0.05$.

Mint has a very high commercial value at present, and will be applied to develop more products in the future. Some studies on soilless culture technology focusing to the development of synthetic substrates were reported (Jensen and Collins, 1985; Papadopoulos, 1986). For example, glass fiber, rock wool and polyurethane foam were applied in soilless culture (Benoit and Ceusternans, 1996; Hardgrave, 1995). However, because of the high cost of equipment, its application was limited (Boodey, 1984). Nutrient solution, especially in Yamazaki formula has a positive effect on plant growth and development, but was less reported in mint (Papadopoulos, 1986). In the current study, we systematically studied the effects of different concentration of nutrient solution on the growth and development of mint, and explored the most suitable nutrient solution for soilless cultivation of mint.

MATERIALS AND METHODS

Mint seeds were planted in greenhouse in the experimental base of forest and fruit building of Guangdong Ocean University. Japanese Yamazaki method was used for nutrient solution consisting of $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ (236 mg L^{-1}), KNO_3 (404 mg L^{-1}), $\text{NH}_4\text{H}_2\text{PO}_4$ (57 mg L^{-1}), and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (123 mg L^{-1}) as described previously (Guo, 2011), four nutrient solution concentrations were set up for treatments, namely 0.5x, 0.75x, 1.0x and 1.25x, and treatments of microelements were referred to by Guo (2011). The general formula for trace elements include EDTA-NaFe (30 mg L^{-1}), H_2BO_3 (2.86 mg L^{-1}), $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ (2.13 mg L^{-1}), $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ (0.22 mg L^{-1}), $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ (0.08 mg L^{-1}) and $(\text{NH}_4)_6\text{MO}_7\text{O}_{24}$ (0.02 mg L^{-1}) (Guo, 2011).

This experiment was conducted based on a single factor design, with four treatments of different nutrient concentrations, and 100 mint seedlings were used for each treatment. Three independent biological replicates were conducted. The Japanese Yamazaki formula (Ming et al., 2007) was used as the stock solution to prepare nutrient solution of different concentrations. The four treatments were set up: 0.5x, 0.75x, 1.0x, 1.25x nutrient solution concentrations, and distilled water were used as control. When the mint seedlings grew to about 3 cm, the seedlings of mint seedlings were transplanted to soilless culture in a foam box with river sand

as substrate. Leaves were sampled at different time-points, including the date after transplantation 15 days, 22 days after transplantation, 29 days after transplantation and 36 days after transplantation, respectively. Nutrient solution was irrigated once every three days. At 15, 22, 29 and 36 days after transplantation, 15 samples were collected and washed. Physiological indexes of fresh weight, plant height, stem diameter, root length, leaf width, leaf length and functional leaves were determined. The chlorophyll content and soluble sugar content were determined by anthrone colorimetry [19], soluble protein contents were determined by Coomassie Brilliant Blue G-250 and Vc content were measured according to ammonium molybdate colorimetry (Gao, 2006). Data were analyzed by SPSS and Excel software, and one-way ANOVA was applied according to Duncan method (Liu and Wang, 1999).

RESULTS AND DISCUSSION

Effect of nutrition solution of different concentrations on root length and stem thickness of *Mentha*

Mint is famous for special odor and well-known for its values in medicinal functions and edible (Xu, 2007). Therefore, the demand for mint will be definitely increasing in the future. Morphological traits such as plant height, stem diameter, root length, leaf length, leaf width and fresh weight of mint are reflective to the growth and development of mint in various nutrient solution concentration (Shen et al., 2019). As shown in Figure 1A, the root length of 0.75x treatment was significantly different from that of control (defined as 1x nutrition concentration) at 15 days after transplantation, but the other three treatments were not significantly different from that of control, suggesting that optimized nutrition solution could promote the growth and development of Mint root length, which is consistent with conclusion reported in other study (Li et al., 2011). Except 1.25x treatment, the root length of other treatments is slightly shorter than that of control; all other treatments promote root growth. At 22 days after transplantation, the root length of 0.75x treatment was significantly different from that of other

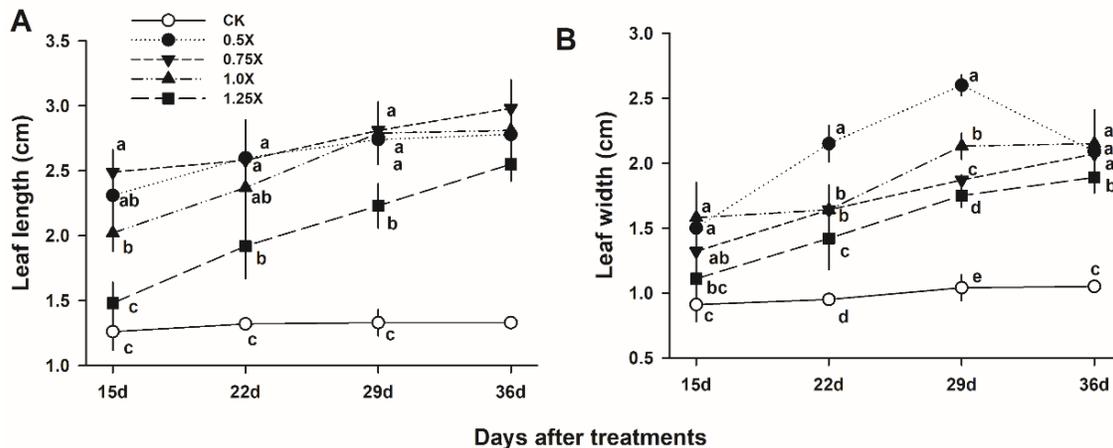


Figure 2. Effects of nutrient solutions concentrations on leaf structure of *Mentha Mint*. A-B, represent leaf length and width. Different alphabetic letters in the values of different concentrations for each time point of treatment indicate significant difference at $P < 0.05$.

treatments and controls, following ranking that $1.0x > 0.5x > 1.25x$. At 29 days after transplantation, the root length of 0.75x treatment exhibited the largest difference compared with the control, which increased by 2.19 cm, with an increase of 119%. There was no significant difference between other treatments. For 36 days after transplantation, there were significant differences between the treatment and the control.

As shown in Figure 1B, the stem diameter of each treatment was larger than that of control at 15 days after transplantation. Except for 1.25x treatment, there was no significant difference between the other three treatments and control. For the samples of 22 days after transplantation, the stem diameter of each treatment was significantly different from that of the control. The treatment of 0.75x had the most obvious effect, followed by 0.5x and 1.0x, and the treatment of 1.25x had the obviously negative effects on stem diameter. For 29 days after transplantation, there were significant differences between the treatments and the control, where the effect of 0.75x treatment showed the most obvious effects. The differences between 0.5x, 1.0x and 1.25x treatment were marginal, and the differences between the treatments and the control were significant. In terms of 36 days after transplantation, there were significant differences between treatment and control regarding 0.75x treatment and other three treatments, but no significant differences among the other three treatments. The stem diameter of the control decreased following prolonged treatment duration.

Effects of nutrient solutions with different concentrations on leaf length and width of mint

According to Figure 2A, there were significant differences

in leaf length between 0.75x, 0.5x and 1.0x treatments 15 days after transplantation, but no significant difference was observed between 1.25x treatment and control. However, leaf growth was also increased in 1.25x treatment, and leaf growth was promoted across all treatments. For 22 days after transplantation, there were significant differences between the treatments and the control. There were no significant differences between the treatments of 0.5x, 0.75x and 1.0x, and the effects of the three treatments were more obvious than that of 1.25x. After 29 days of transplantation, the growth rate of leaf length was accelerated in 0.75x treatment. When 36 days after transplantation, the leaf length of 0.75x treatment was significantly longer than that of control and the increase was 1.48 cm, but there was no significant difference among the other three treatments.

As shown in Figure 2B, the leaf width of 0.75x, 0.5x and 1.0x treatments at 15 days after transplantation was significantly different from that of the control, but there was no significant difference between 1.25x treatments and the control. The leaf width of 1.25x treatment was wider than that of the control. The leaf width and length were increased across all treatments. For 22 days after transplantation, there were significant differences between the treatments with different concentrations and the control, but there were no significant differences between the treatments with 1.25x, 0.75x and 1.0x, and the leaf width of 0.5x exhibited the largest compared to other treatments. Regarding 29 days after transplantation, the difference between treatments was similar to 22 days after sowing.

When treating 36 days after transplantation, there were significant differences between the treatments and the control, but there was no significant difference between the treatments. Compared with the control group, the leaf width values of mint for 1.0 x concentration increased

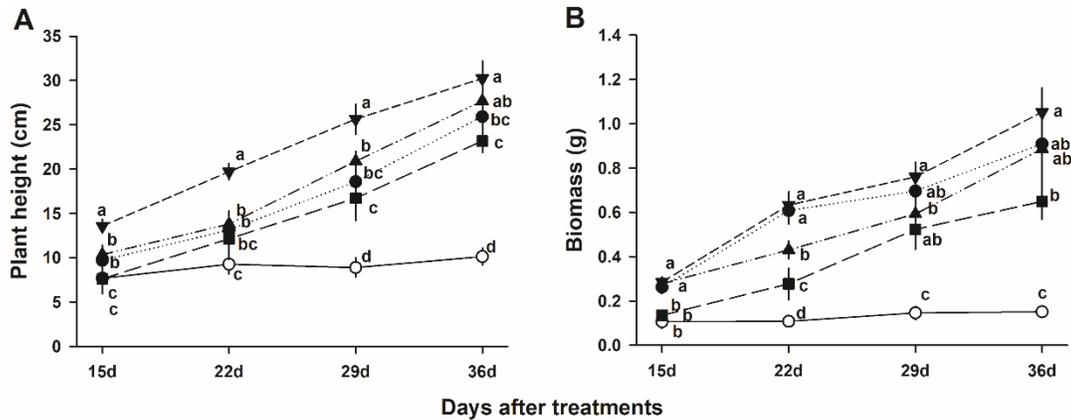


Figure 3. Effects of nutrient solutions concentrations on canopy morphology of *Mentha Mint*. A-B, represent plant height and biomass. Different alphabetic letters in the values of different concentrations for each time point of treatment indicate significant difference at $P < 0.05$.

by 1.10 cm.

Effects of different concentrations of nutrient solution on plant height of mint

Plant height of 0.75x and 1.0x treatments at 15 days after transplantation was significantly different from that of the control (Figure 3A). The plant height of the other two treatments was not significantly different from that of the control. The values of mint plant height at 1.25x were slightly lower than that of the control (1.0x). For 22 days after transplantation, there were significant differences between the treatments and the control, 0.75x and the other three treatments, 1.0x > 0.5x > 1.25x, and 1.25x increased significantly. In terms of 29 days after transplantation, the plant height of mint for each treatment was significantly different from that of the control, where plants in 0.75x of concentration showed the most significant differences, and plants in 1.0x nutrition treatment exerted the fastest growth rate, with an increase rate of 151.4%. When treating 36 days after transplantation, there were significant differences between the treatments and the control.

The difference of plant height between 0.75x and the control reached the maximum value, with a difference of 20.10 cm. It also showed significant differences for other three treatments with the control. These findings indicate that nutrient solution plays a crucial role in promoting the growth of plant height, which support the observation of enhanced growth characteristics treated with nutrient solution in some hydroponic plants (Chen, 2017).

It was reported that nutrient solution has a significantly positive effect on the growth and development in some horticultural species, such as cucumbers (Li et al., 2011). According to Figure 3B, the values of mint fresh weight for 0.75x, 1.0x and 0.5x treatments at 15 days after transplantation were significantly different from that of

control, but there was no significant difference between 1.25x treatment and control. The values of mint fresh weight for 1.25x treatment were slightly higher than that of control, indicating that nutrient solution was helpful to increase fresh weight. At 22 days after transplantation, there were significant differences between 0.75x and 0.5x treatments and the other two treatments as well as the control. The growth rate of fresh weight of 0.75x > 0.5x, while growth rates of mint for 1.25x treatment was the slowest, and there were significant differences between the treatments and the control. At 29 days after transplantation, the fresh weight of mint for 0.75x treatment were the largest compared to other treatments, but the growth rate of mint for 0.75x treatment slowed down. During the transplantation period, the growth rate of mint fresh weight was 1.25x, and the growth rate reached 188.8%. When treating 36 days after transplantation, there was no significant difference between 0.75x, 1.0x and 0.5x treatments. The ranking of mint fresh weight for different nutrition treatments is: 0.75x > 0.5x > 1.0x, while the value of fresh weight for 0.75x treatments was 9.001 g, which is higher than that of the control.

Effect of nutritional solutions concentrations on soluble sugar content and soluble protein content of *Mentha*

Generally, high soluble sugar content is favorable for foliar flavor. Study from Li et al. (2011) suggested that proper concentration of nutrient solution could enhance soluble sugar content in vegetable fruits and improve the quality of cucumber. The main element affecting the content of soluble sugar is phosphorus (Su et al., 2014). According to Figure 4A, for 15 days after transplantation, there were significant differences regarding the soluble sugar content between each treatment and the control,

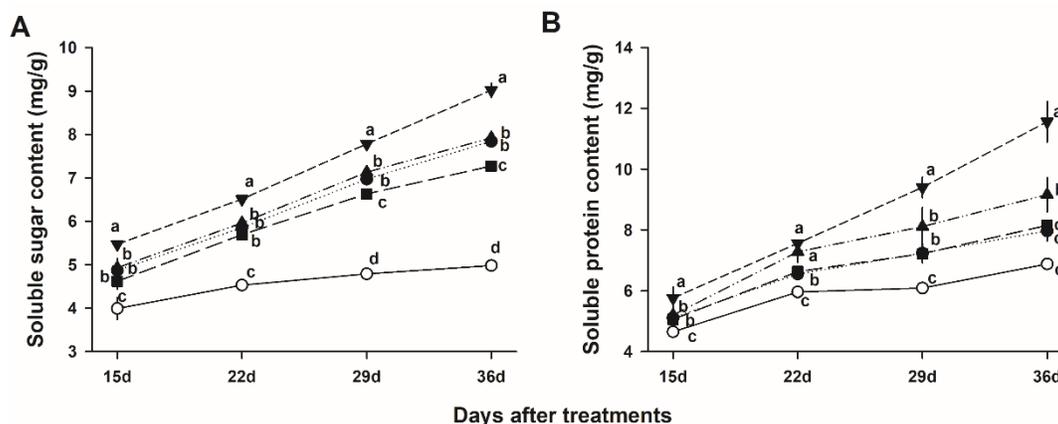


Figure 4. Effects of nutrient solutions concentrations on soluble sugar content and soluble protein content of *Mentha Mint*. A-B, represent soluble sugar content and soluble protein content. Different alphabetic letters in the values of different concentrations for each time point of treatment indicate significant difference at $P < 0.05$.

while there was no significant difference between 1.0x and 0.5 x treatments. In addition, there were no significant differences between 1.25x and 0.5x treatment and other treatments; the soluble sugar content of 1.25x and 0.5x treatment was not significantly different: 0.5x > 1.25x. At 22 days after transplantation, the soluble sugar content of 0.75x treatment was significantly higher than that of the other three treatments and the control, suggesting that undesirable concentration of nutrient solution would reduce the soluble sugar content. The results in this study are in agreement with the conclusion by Su et al. (2014), that the soluble sugar content of lettuce can be increased by the appropriate concentration of phosphorus.

The other three treatments were significantly different from the control, the ranking of effects on sugar content is: 1.0x > 0.5x > 1.25x > CK. In terms of 29 days after transplantation, the soluble sugar content of each treatment was significantly different from that of the control, and there were significant differences among the treatments, the ranking changed to: 0.75x > 1.0x > 0.5x > 1.25x > CK. At 36 days after transplantation, the soluble sugar content of 0.75x treatment increased by 2.94 mg/g as compared with the control group. The soluble sugar contents for other three treatments were significantly higher than the control group. Therefore, the soluble sugar content for each treatment of nutrient solution was increased compared with the control, and the soluble sugar content of plants treated with nutrient solution was increased by 45.98 to 81.12%, compared with the control, suggesting the soluble sugar content is probably insensitive to nutrition solution.

Soluble protein content is also an important nutritional index of vegetables (Kučerová et al., 2018). In early stage of growth and development of Mint, the difference of soluble protein content among different nutrient solution

treatments was not very large (Figure 4B). Following longer treatment, we found that the high concentration of nutrient solution significantly promoted soluble protein content of the leaves. This probably indicates that the synthesis of soluble protein were inhibited by extremely high concentration of nutrient solution (Zhang et al., 2019), and the soluble protein content of the plant treated with 0.75x medium concentration was inhibited. The difference between 0.75x treatment and the other three treatments was the most significant for other treatments, while the corresponding ranking is: 1.0x > 0.5x > 1.25x.

At 22 days after transplantation, the soluble protein content of 0.75x treatment was significantly higher than that of the other three treatments and controls. The soluble protein content of 1.0x treatment increased up to 2.08 mg/g, which is the highest than that of other treatments. The difference between 1.0x and 0.5x and 1.25x was significant, but there was no significant difference between 0.5x and 1.25x treatment. In terms of 29 days after transplantation, there was no difference in soluble protein content between the treatments and the control ranking is: 0.75x > 1.0x > 0.5x > 1.25x > CK. At 36 days after transplantation, the difference of soluble protein content between 0.75x treatment and control reached the maximum of 4.68 mg/g; the other three treatments were significantly higher than the control, while the corresponding ranking is: 1.0x > 1.25x > 0.5x. During the period, the soluble protein content of 1.25x treatment increased by 0.93 mg/g, which is exceeding over 0.5x treatment.

Therefore, appropriate nutrient concentration is helpful to increase the soluble protein content of plants, and excessive nutrient concentration will play an inhibitory role. This is in line with other study that reported that the soluble protein showed a decreasing trend with the increase of nutrient solution concentration (Wang, 2016).

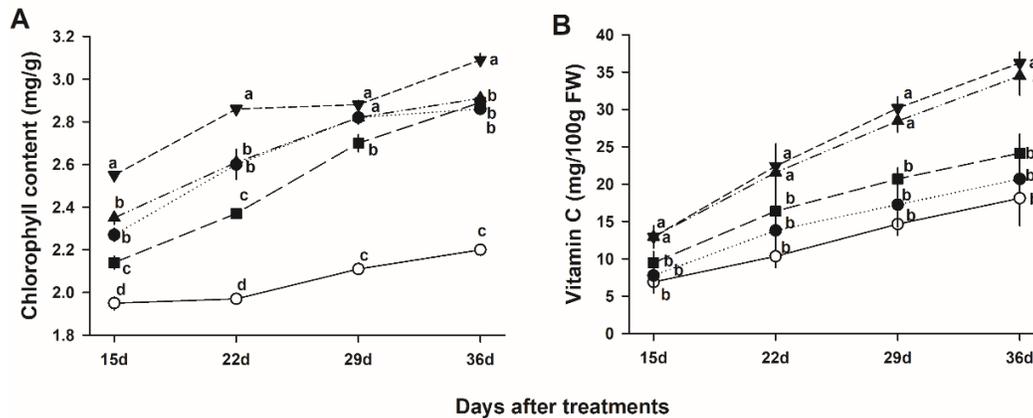


Figure 5. Effects of nutrient solutions concentrations on soluble sugar content and soluble protein content of *Mentha* Mint. A-B, represent chlorophyll content and vitamin C content. Different alphabetic letters in the values of different concentrations for each time point of treatment indicate significant difference at $P < 0.05$.

Effects of different concentration of nutrient solution of mint

Leaf chlorophyll content is a key factor to indicate photosynthetic capacity. According to Figure 5A, the chlorophyll content of each treatment was significantly different from that of the control. For 15 days after transplantation, the ranking of chlorophyll content for different nutrition concentration was: $0.75x > 1.0x > 0.5x > 1.25x > CK$. At 22 days after transplantation, the chlorophyll content of mint for 0.75x treatment remained significantly higher than that of the other three treatments and the control. The chlorophyll content of 0.5x treatment was not significantly different from that of 1.0x treatment. For 29 days after transplantation, there were significant differences between each treatment, the corresponding ranking is: $0.75x > 1.0x > 0.5x > 1.25x > CK$. At 36 days after transplantation, the values of mint chlorophyll content at 0.75x showed the greatest difference compared with the control. There was no significant difference between 0.5x, 1.0x and 1.25x treatments.

Across different concentrations, the chlorophyll content of all treatments continued to increase with prolonged duration. The chlorophyll content of CK treatment decreased on the 36th day after transplantation, which reveals that nutrient solution could promote the synthesis of chlorophyll. Chlorophyll content of mint leaves, observed in the present study, were increased across all concentration of nutrient solution (Figure 5A), which is consistent with results reported by other study (Cheng, 2013). There were significant differences regarding chlorophyll *a* and chlorophyll *b* content between treatments with different concentrations and control. The chlorophyll content was increased in all treatments, indicating that nutrient solution was helpful to promote chlorophyll synthesis. The content of vitamin C constitutes

to an important index to determine the quality of vegetables and fruits. As indicated in Figure 5B, plants at 15 days after transplantation, the contents of vitamin C in 75x and 1.0x treatments were significantly different. Ranking of vitamin C contents for different nutrition treatments were $1.25x > 0.5x > CK$. At 22 days after transplantation, the contents of vitamin C for the 0.75x treatment were not significantly different from that for 1.0x treatment. The content of vitamin C for the 0.75x treatment was slightly higher than that for 1.0x treatment, and the difference between 1.25x treatment and control was significant. This is consistent with the conclusion reported from other study (Qin, 2017). For 29 days after transplantation, the pattern remained unchanged, 0.75x, 1.0x and 1.25x were significantly different from the control, while the corresponding ranking is: $0.75x > 1.0x > 1.25x > 0.5x > CK$. At 36 days after transplantation, the difference of vitamin C content between 0.75x treatments and control reached up to 18.10 mg/100 g fresh weight, while the vitamin C content for both 1.0x treatment and 1.25x treatments were significantly different from the control, while the difference of vitamin C content between 0.5x treatment and control was not significant.

Conclusion

Overall, this study provides a systematic survey on the dynamic effects of different nutrition concentration on physiological and biochemical parameters in mint seedlings. It was concluded that 0.75x nutrient solution constitutes the most suitable concentration for the growth and development of menthol in soilless cultivation, and the optimized nutrition concentration guides the solution for future plant factory practice and vertical farming measures.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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