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EFFECTS OF FOLIAR-SPRAYED FERMENTED JAPANESE HOP (*HUMULUS SCANDENS* MERR.) JUICE ON THE GROWTH AND YIELD OF LETTUCE

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Abstract

To explore the effect of Fermented Japamese Hop Juice, lettuce leaves were sprayed with different concentrations of the juice, and then their effects on lettuce growth, morphology, and nutritional quality were analyzed. The results showed that, compared to control check (CK), the concentrations of 300 times diluted extract (treatment D) increased the dry weights, fresh weights, the contents of soluble sugar, soluble protein, Vc and anthocyanin of lettuce by 17.78, 12.37, 13.64, 42.67, 21.21 and 78.57%, respectively. Additionally, the activity of SOD and CAT increased by 89.78 and 50%, respectively. On day 28 of treatment D, the net photosynthetic rate and intercellular carbon dioxide concentration increased by 12.44 and 21.76%, respectively and transpiration rate stomatal conductance decreased 19.58%. The results strongly suggest that appropriate concentration of the Fermented Japamese Hop Juice could effectively promote the growth of lettuce, increase the activity of antioxidant enzymes, and improve the photosynthetic performance, especially for improving the quality of lettuce. Overall, foliar-sprayed diluted 300 times Fermented Japamese Hop Juice extract is suitable concentration for lettuce production applications.

Introduction

Plant enzymes which contain various organic and inorganic compounds have become popular in Japan, the United States, some countries in Europe, Southeast Asia and Taiwan (Zhu *et al.* 2011, Jiang 2013, Li *et al.* 2014). It is reported that plant extracts can be extracted from natural plant materials or economic crops by fermentation, and are beneficial for the improvement of plant growth (Wu *et al.* 2016), root vigor (Anita *et al.* 2016), fruit quality (Dai *et al*. 2008), and disease resistance (Wei *et al.* 2009, Geng *et al.* 2011). It was found that foliar application of the herb nutrient solution could promote the growth of new shoots (Liu *et al.* 2011). In addition, the result showed that foliar-sprayed with the concentration of 200 times pear fruit-derived nutrient solution can significantly enhance the net photosynthetic rate and water use efficiency of leaves (Luo *et al.* 2012). Furthermore, plant enzymes have been forming standardized production technology in Korea, Japan, Malaysia, and so on (Guo and Zhao 2014, Fang *et al*. 2017, Sakimin *et al*. 2017).

Japamese Hop is a kind of drought-resistant and poor-tolerant plant. Previous studies have shown that it contains various nutrients such as protein, amino acids, vitamins, and also contains macroelements, including nitrogen, phosphorus, potassium, and microelement, such as iron (Dong 1999, Yin *et al*. 2001, Wang *et al*. 2005, Duan *et al.* 2006, Feng and Qi 2009). Beside the importance on plant, it also plays a role in anti-inflammatory (Hou *et al.* 2009), antibacterial (Lv *et al*. 2009), anti-oxidation (Pandurangan and Ganapasam 2008) in animal life and clinical application of medicine (Liang 2000, Li and Ren 2003, Cai and Wang 2004). Moreover, its extracts also have been proved for the improvement of seed germination and seedling growth (Cai and Wang 2004, Ou and Gao 2011). The aims of the present study were to investigate the

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influence of foliar-sprayed Fermented Japamese Hop Juice (FJHJ) on growth, physiology, yield, quality of hydroponic lettuce, and provide technical reference and theoretical basis for the comprehensive application and development of plant-derived organic nutrient solution.

Materials and Methods

The experiment was conducted in a plastic greenhouse covered by a double membrane in the third district of Henan Agricultural University from November 11 to December 16, 2017.

Lettuce (*Lactuca sativa* L., Italian) plants were provided by the Yifenghe Seed Company and were chosen as plant material for further experiments. The plants were sprayed with FJHJ at different concentrations at 7 days after planting (DAP) and repeated the application every 7 days for 4 times. The concentrations of FJHJ were diluted 50 times (A), 100 times (B), 200 times (C) and 300 times (D), the control plants (CK) were applied with water. Each treatment had three replications and each of the three replicates had twelve plants. During the whole growth period, every day the nutrient solution was supplied from 7:00 to 19:00 for 15 min every hour. Besides, it was supplied at 24:00 and 6:00 for 15 min, respectively. When the temperature was lower than 5ºC at night, it was warmed by the hotline placed under the cultivation frame at 18:00 - 7:00. Then selected sunny days determined photosynthetic index at 9:00 to 11:00 after planted 20 days, once every 7 days for 3 times. After 35 days of colonization six plants were sampled randomly from each group and plant high (cm), stem diameter (mm), longest root (cm), shoot fresh weight (g) were measured (Table 3). Soluble sugar content (g/100g), soluble protein (mg/g), Vc content (g/100g), anthocyanin content (unit/g); SOD (u/g), CAT (u/g*min), POD (u/g*min) and photosynthetic parameters were measured using the methods described by Li (2000).

FJHJ was prepared by a combination of aerobic fermentation and anaerobic fermentation. The fermentation substrate were (W): Japamese Hop 13.5%, brown sugar 5.4%, soybean meal 0.06%, potassium feldspar powder 0.035%, phosphorite powder 0.035%, water 80.96%. The length of stems and leaves of fermented Japamese Hop was 2 to 3 cm and was Stirred once every 3 - 5 days when aerobic fermentation, then after 25 days when the pH value and EC value were steady, taking anaerobic fermentation, diluted it after 20 days and applied it on the basis of pre-test .The composition of the FJHJ is shown in Table 1. Hoagland nutrient solution was shown in Table 2.

Table 1. The content of primary substance and physico-chemical properties in the FJHJ.

Composition	Total N (mg/l)	Total P (mg/l)	Total K (mg/l)	Soluble solids (%)	Soluble sugar (mg/l)	pH	EC (ms/cm)
Content	1177.5	103.69	696.71	3.20	9132.43	5.62	>1

Results and Discussion

The plant height, stem diameter, and shoot fresh weight of lettuce initially decreased and then increased with the increase of FJHJ (Table 3). The plant height, stem diameter, and the shoot fresh weight of the treatment D were significantly higher than those of treatment B and C, but there was no significant difference compared to treatment A and CK. Besides, the maximum root length and shoot dry weight of Treatment D were significantly higher than those of other treatments and CK, and they were, 31.63 and 17.78%, respectively higher than those of CK. These indicated that the FJHJ could promote the growth of lettuce roots and increase the accumulation of dry matter in shoots.

Table 2. Hoagland formulation as a stock of chemical fertilizer used in the experiment.

Stock	Type of fertilizer	Composition (mg/l)
A	Calcium nitrate[$Ca(NO_3)_2$]	589.2
	Potassium nitrate[KNO_3]	508.1
B	Potassium dihydrogen phosphate [KH_2PO_4]	132.9
	Ammonium nitrate [NH_4NO_3]	80
	Magnesium sulfate [$MgSO_4$]	182.5
	Nitric acid [HNO_3]	0.267
C	Iron EDTA [Fe]	16
	Boric acid [H_3BO_3]	3.5
	Manganese sulfate [$MnSO_4$]	0.2
	Zinc sulfate [$ZnSO_4$]	0.22
	Copper sulphate [$CuSO_4$]	0.08
	Ammonium molybdate [$(NH_4)_6Mo_7O_{24}.4H_2O$]	0.5

Statistical analysis was carried out with SPSS 17.0 (Spss Inc., Chicago, USA) and the means were separated using the Least Significant Difference (LSD) test at $p < 0.05$.

Table 3. The influence of different treatments on the growth of lettuce.[a]

Treatment \ Indicators	Plant high (cm)	Stem diameter (mm)	Root length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)
CK	10.30±0.15ab	8.88±0.42a	31.3±0.87b	29.58±2.03ab	1.80±0.14b
A	10.13±0.27ab	8.62±0.56a	28.0±0.88b	29.54±1.3ab	1.81±0.05b
B	9.70±0.25b	7.31±0.18b	25.4±0.81b	24.70±1.52c	1.82±0.10b
C	9.63±0.19b	7.33±0.32b	26.8±0.44b	28.14±0.42bc	1.75±0.05b
D	10.67±0.44a	9.34±0.38a	41.2±0.60a	33.24±0.83a	2.12±0.06a

[a] Mean values and ± SE with different lower case letters in a row are significantly different at $p < 0.05$.

The soluble sugar and Vc content of lettuce increased initially and then decreased with the increase of the dilution multiple of FJHJ (Fig.1a, 1c). The soluble sugar content of C and D were both higher than CK, and they did not reach significant levels (Fig.1a). The contents of Vc in treatment C and D were significantly higher than that in CK, which were 31.82 and 21.21%, respectively higher than the control, respectively (Fig.1c). The soluble protein content and the anthocyanin relative content of lettuce increased with the increase of the dilution times of FJHJ, and not only both of them were higher than that of CK, but also the differences were significant (Fig.1b,d). Besides, the content of soluble protein and the relative content of anthocyanin in treatment D were 42.67 and 78.57%, respectively higher than that of CK. These indicate that the appropriate concentration of FJHJ can significantly improve the quality of lettuce.

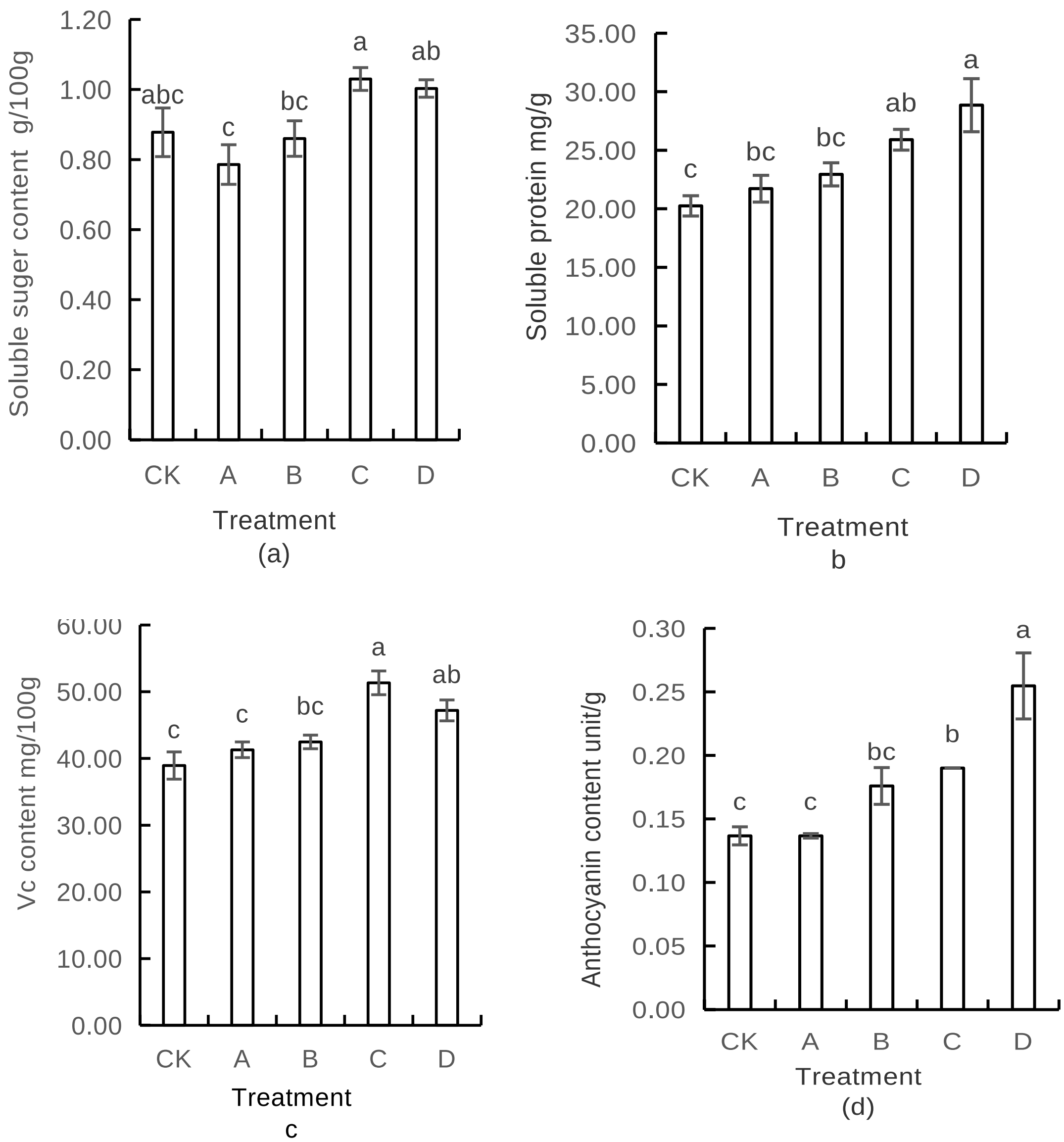

Fig. 1. Quality of lettuce with different treatments. (a) Soluble sugar content, (b) Soluble protein, (c) Vc content and (d) anthocyanin content.

Changes in antioxidant enzyme activities were mainly dependent on ROS content. Compared to CK, the activity of SOD significantly increased (89.78%) in lettuce under treatment D and there was no significant difference under other treatments (Fig. 2e). In addition, the significantly increase in activity of CAT was induced 27.12 and 50% by treatments C and D, respectively (Fig. 2f). Nevertheless, the activity of POD significantly decreased under FJHJ treatments, compared to CK (Fig. 2g). It showed that the suitable concentration of FJHJ could increase the activity of lettuce SOD and CAT, and the treatment D works best.

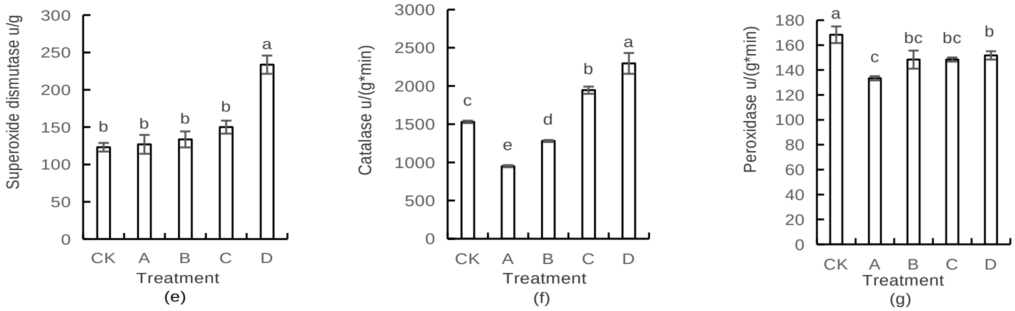

Fig. 2. Protective enzyme system of lettuce with different treatments. (e) Superoxide dismutase (SOD, u./g), (f) catalase (CAT , u/(g*min)) and (g) peroxidase (POD, u/(g*min)).

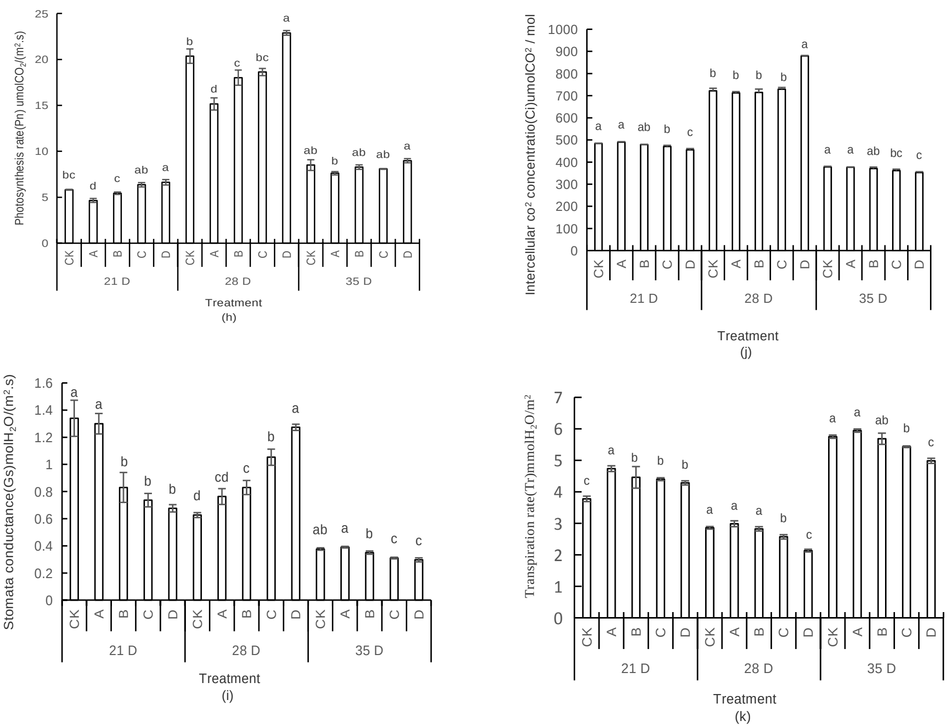

Fig. 3. Photosynthetic parameters with different treatments in different stages of lettuce. (h) photosynthetic rate (Pn), (i) stomatal conductance (Gs), (j) intercellular carbon dioxide concentration(Ci), (k) transpiration rate (Tr).

The photosynthesis rate (Pn) of each treatment in each period increased with the increase of the dilution multiple of FJHJ, and the Pn in each period of treated D was higher than that of CK, the 21 and 28 days' Pn were significantly 13.92 and 12.44%, respectively higher than CK (Fig. 3h). The 21 and 35 days' stomata conductance (Gs)and intercellular carbon dioxide concentration (Ci) decreased with the increase of the dilution multiple of the FJHJ, both Gs and Ci showed an upward trend at 28 days, and the Gs and Ci of treated D were significantly different from CK in each period (Fig. 3i, j).The transpiration rate (Tr) of each treatment in each period decreased with the increase of the dilution multiple of FJHJ, and the Tr in each period of treatment D was significantly different from that of CK, it was 25.4% higher than that of CK at 21 days, 19.58 and 13.39% lower, respectively than those of CK at 28 and 35 days (Fig. 3k). These indicate that the appropriate concentration and use time of FJHJ can increase the photosynthetic performance of lettuce. What's more, treatment D has the best effect at 28 days, which can significantly increase Pn, Gs, Ci and significantly reduce Tr of lettuce.

Main findings of the present experiment are: (1) the suitable concentration of FJHJ promotes the growth and the shoot's dry matter accumulation of lettuce. Compared to CK the effect of treatment D is the best, their maximum root length, the shoot's fresh and dry weight increased by 31.63, 12.37 and 17.78%, respectively. (2). The appropriate concentration of FJHJ could significantly improve the quality of the lettuce and the effect of treatment D is better, their soluble sugar content, soluble protein content, Vc content and anthocyanins relative content were 13.64, 42.67, 21.21 and 78.57%, respectively higher than CK.(3). The FJHJ with appropriate concentration can improve the activity of SOD and CAT of lettuce, among which the effect of treatment D is the best, and the activity of SOD and CAT increased by 89.78 and 50%, respectively compared with CK.(4). Appropriate concentration and the use time of FJHJ can improve the photosynthetic performance of lettuce, among which the effect of treatment D is the best for the 28 days' lettuce, their Pn and Ci significantly increased by 12.44 and 21.76%, respectively than CK, the Tr of them decreased significantly by 19.58%. In conclusion it may be said the suitable concentration of FJHJ is beneficial to promote the growth of lettuce, increase the activity of antioxidant enzymes, improve photosynthetic performance, and especially to improve the quality of the lettuce. So the application of foliar fertilizer with FJHJ diluted 300 times is feasible.

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