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Full Length Article

Yield and Nutritional Quality of Water Spinach (*Ipomoea aquatica*) as Influenced by Hydroponic Nutrient Solutions with Different pH Adjustments

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Abstract

It is critical to identify effective buffer chemicals which are capable of regulating the pH of nutrient solution to a desirable level for best hydroponic production of crops. Greenhouse experiments were conducted to examine the pH dynamics of nutrient solutions amended with different inorganic acids during hydroponic production and the yield and nutritional quality of the resulting crops. A typical nutrient solution (pH 8.2) was adjusted to a pH of 5.6±0.2 with 1 M HNO_3, 1 M H_3PO_4, 1 M H_2SO_4, and a 3:1:1 (v/v/v) mixture of all three acids, respectively. Water spinach (*Ipomoea aquatica* Forsk) was hydroponically grown for 25 d in the unadjusted control and the pH-adjusted nutrient solutions. The different treatments were monitored for pH changes of the nutrient solution, and measured for shoot yield and nutritional quality of the grown water spinach. It showed that the solution pH adjustments introduced additional anions but did not significantly increase the electrical conductivity (EC). The HNO_3-H_3PO_4-H_2SO_4 mixture was able to achieve an optimal solution pH ranging from 5.5 to 6.5, while any of the acids only failed to maintain the solution pH within optimal range for 48 h after each adjustment. The shoot fresh weight, dry weight, and height of water spinach grown in the mixed acids-treated solution were the greatest among the five treatments. Relative to the control, the acid mixture treatment also increased the vitamin C, soluble sugar and crude protein contents in plants. Thus, the mixed 3:1:1 (v/v/v) HNO_3-H_3PO_4-H_2SO_4 is recommended for regulating the pH of nutrient solution in hydroponic production of leafy greens.

Keywords: Hydroponics; pH; Acids; Nutrient solution; Water spinach

Introduction

Hydroponics, a method for growing crops in nutrient solutions instead of mineral soils, has been increasingly practiced in horticultural production due to its high water, nutrient, and labor efficiencies (Rius-Ruiz *et al.*, 2014; Aftab *et al.*, 2015). The chemical composition of hydroponic solution can be readily adjusted to meet the specific requirements of particular crops for nutrients (Caruso *et al.*, 2011). Typically a nutrient solution contains all essential plant nutrients in water soluble forms and in appropriate concentrations. Nevertheless, the supply buffering capacity of a nutrient solution is nearly null (Gorbe, 2009), leading to high danger of hydroponic plants to nutrient deficiency and ion toxicity (Gorbe and Calatayud, 2010).

The pH of hydroponic solution is a major factor affecting nutrient availability and plant uptake (Spinu *et al.*, 1997). The existing forms (speciation) and subsequently the phytoavailability of a nutrient element in the solution are greatly influenced by solution pH. For example, increasing pH to above 7.3 would change the predominant form of P in solution from $H_2PO_4^-$ to HPO_4^{2-}. In an alkaline solution, Ca^{2+}, Mg^{2+}, Fe^{3+}, Cu^{2+}, and other polyvalent cations would form precipitates with OH^- and CO_3^{2-} and become unavailable to plants. In strongly acidic solutions, H^+ competes with other mineral nutrients for plant absorption (Brady and Weil, 2007), to ensure the best availability of essential nutrients to hydroponic plants, the pH of a nutrient solution should be maintained in a narrow range usually between 5.5 and 6.5 (Webb, 1993). Nevertheless, the pH of a nutrient solution may change during hydroponic production owing to selective absorption of soluble ionic nutrients by plants (Kim *et al.*, 2005). To absorb cation and anion nutrients, plant roots release H^+ and HCO_3^-, respectively, into the solution for exchange, causing shifting of the solution pH over time (Brady and Weil, 2007).

The commonly used pH regulators are inorganic acids such as nitric acid (Wheeler *et al.*, 1990; Webb, 1993; Savvas and Adamidis, 1999; Savvas *et al.*, 2006), hydrochloric acid (Domingues *et al.*, 2012), and sulfuric

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acid (Massa *et al.*, 2011; Giuffrida and Leonardi, 2012). Addition of HNO_3 provides extra N nutrient in the form of NO_3^-. Due to chloride ions are harmful to most crops (Bernstein, 1975), hydrochloric acid (HCl) cannot be used for hydroponic solution pH adjustment. Use of sulfuric acid (H_2SO4) and phosphoric acid (H_3PO_4) for the purpose has been rarely reported. Little is known about the capability of these inorganic acids in maintaining a relatively stable pH of hydroponic solution and the effects of these acids as a pH adjustment on the yield and quality of hydroponic plants. Furthermore, when a single acid (e.g., HNO_3) is used to regulate the solution pH, the concentration of the acid radical anion (e.g., NO_3^-) in the nutrient solution will increase and become excessive for hydroponic plants (Prasad *et al.*, 2001; Bugbee, 2004). This study aimed to identify a practical buffer agent capable of effectively regulating the pH level of hydroponic nutrient solution and promoting plant growth. To this end, we examined the pH dynamics of nutrient solutions amended with different inorganic acids during hydroponic production and the yield and nutritional quality of the crops.

Materials and Methods

Preparation of Hydroponic Nutrient Solution

A nutrient solution was prepared following the formula of modified half-strength Hoagland solution. The nutrient solution contained NO_3^--N 42 mg L^{-1}, SO_4^{2-}-S 32 mg L^{-1}, $H_2PO_4^-$-P 31 mg L^{-1}, K^+ 78 mg L^{-1}, Mg^{2+} 24 mg L^{-1}, Ca^{2+} 40 mg L^{-1}, Fe^{2+} 2800 µg L^{-1}, Cu^{2+} 20 µg L^{-1}, Zn^{2+} 50 µg L^{-1}, Mn^{2+} 500 µg L^{-1}, $B(OH)_4^-$ -B 500 µg L^{-1}, and MoO_4^{2-}-Mo10 µg L^{-1}. Analytical grade chemicals were dissolved in underground water to make the desired elemental concentrations of the nutrient solution.

The nutrient solution had originally pH of 8.2. It was further adjusted to pH 5.6±0.2 with different inorganic acids. According to the acids used for pH adjustment, five treatments of nutrient solutions were generated: 1) Control – 80 L of the original nutrient solution without any pH adjustments; 2) HNO_3 – 80 L of the original nutrient solution were adjusted to pH 5.6±0.2 using 1 M HNO_3; 3) H_2SO_4 – 80 L of the original nutrient solution were adjusted to pH 5.6±0.2 using 1 M H_2SO_4; 4) H_3PO_4 – 80 L of the original nutrient solution were adjusted to pH 5.6±0.2 using 1 M H_3PO_4; and 5) Mixed Acids – 80 L of the original nutrient solution were adjusted to pH 5.6±0.2 using mixed 3:1:1 (v/v/v) 1M HNO_3:1M H_2SO_4: 1M H_3PO_4.

Hydroponic Growth of Water Spinach

Water spinach (*Ipomoea aquatica* Forsk cv. bamboo leaf) seeds were procured from a local seed company in Beijing, China. Prior to germination, the seeds were treated with 0.5% (w/w) K_2MnO_4 solution for 0.5 h to destroy any pathogens, rinsed triply with deionized water and soaked in saturated $CaSO_4$ solution at 30°C for 4 h. The pre-treated seeds were then germinated by placing them on a moist filter paper in darkness at 25°C for 24 h. The germinated seeds were then transferred to identically ten trays of sponge cubes, irrigated with no pH-adjustment nutrient solutions, and grew in an incubation chamber for 14 days prior to transplanting.

Five hydroponic growth channels were prepared, each containing one of the five pH-adjustment nutrient solutions, respectively. The growth channels were 500 cm (length) × 50 cm (width) × 12 cm (height) NFT-PVC (nutrient film technology-polyvinyl chloride) gullies acquired from Beijing vegetable research center. Each growth channel was covered with five polyfoam boards (100 cm × 50 cm) each carrying 49 plantation holes (3 cm in diameter). The growth channels were located in a solar greenhouse at the National Experimental Station for Precision Agriculture, Beijing, China. A 60-L PVC tank was stationed underneath each of the growth channels to contain the nutrient solutions. A hydraulic pump (Model 4500A, Haili Pump Company, Guangdong, China) was installed to each tank to circulate nutrient solution between the tank and the channel. The 14-d water spinach seedlings corresponding to the nutrient solution treatments were transplanted to the growth channels in the polyfoam boards in rows at 7.5 cm in-row spacing and 15 cm between-row spacing. In each polyfoam board 16 seedlings were planted. A total of 80 seedlings were transplanted in each growth channel. The recirculating pump was operated for continuously 15 min every hour. The nutrient solutions in the hydroponic systems were refilled to their original volumes (~80 L after recirculation) in the tanks every three days during the experiments. The water spinaches were grown for 25 days before harvesting.

Analytical Measurements

Air temperature in the greenhouse was recorded every 30 min using an automatic recording thermometer (model STR30, Senxte Company, Tianjin, China) during the hydroponic experiments. The pH of the nutrient solutions were monitored every 2d at 10:00 am using a portable pH meter (model PHJ-90B, Beijing Hangtian Computer Co., Beijing, China). If a reading was greater than 6.5, the inorganic acid corresponding to the treatment (except Control) was immediately added to bring the solution pH back to 5.6±0.2. The volumes of the acids added were recorded. No efforts were made to regulate the pH of the Control nutrient solution.

To examine the pH and nutrient dynamics of hydroponic solution as a function of plant growth (nutrient absorption), an intensive 3-d monitoring of the nutrient solution chemistry was carried out 21 days (3 weeks) after transplanting. Every 2 h from 9:00 am of the start day, a 40 mL sample was collected from each of the five treatment systems. The samples were immediately measured for pH using the portable pH meter and for electric conductivity

(EC) using a conductivity meter (model DDS-307, Shanghai Precision and Scientific Instrument Inc., Shanghai, China). Aliquots of the samples were measured for NO_3^- concentrations using an ion selective electrode (model PNO_3-1, Shanghai Guangdian Device Factory, Shanghai, China). The samples were further measured for water soluble phosphate concentrations following the molybdenum blue method (Murphy and Riley, 1962). Concentrations of SO_4^{2-} in interval of 4 h were determined using a spectrophotometer (model 752, Shanghai Spectrum Instruments, Shanghai, China) following the turbidimetric method (Chesnin and Yien, 1951).

After 25 days of hydroponic growth, the water spinach plants were harvested. Individual plants from each of the solution treatment systems were randomly selected, cut off the roots, and measured for plant height and fresh weight. Aliquots of the fresh shoots were dried at constant weight in an oven at 75°C. The dry weight of the plants was recorded. The fresh shoots were further ground into slurries and analyzed for quality parameters. The contents of soluble sugar, vitamin C, and crude proteins were determined following the 2, 6-dichloro-indophenol titration method, the anthrone ethyl acetate colorimetic method and the coomassie brilliant blue G250 dye binding method (Li, 2000), respectively. The NO_3^- content was measured following the salycilic acid method (Li, 2000). The results are expressed on the fresh weight basis of plants.

Statistical Analysis

Analysis of variance (ANOVA) was conducted using the SPSS 19.0 statistical software to evaluate the effect of pH adjustment of nutrient solution on the yield and nutritional quality of water spinach. Sample comparisons by Duncan multiple range tests were used to predict the significance of effect differences between any two treatments. The level of significance was set at 0.05.

Results

Daily Courses of Air Temperature

The 30-min air temperature profiles of the greenhouse were carried out is presented in Fig. 1. The temperature fluctuated between 22 and 38°C, reflecting the diurnal shifting and weather variations. The daily fluctuation pattern raised the maximum temperature at 1:00 pm of the day and declined to the minimum value at 6:00 am. The daily minimum temperature also demonstrated a generally decreasing trend due to the seasonal changes during the experiments (from August to September). The average temperature was 26.4°C during the experimental period.

pH and Nutrient Dynamics of Hydroponic Solution

Fig. 2 illustrates the pH of the nutrient solutions with artificial regulation through inorganic acid addition during the 25-d hydroponic growth of water spinach. For the Control treatment without pH adjustments, the nutrient solution demonstrated a high pH value of approximately 8.2±0.2, not optimal for plant growth. For other treatments, the pH of the nutrient solutions increased rapidly from the initial level of 5.5 to above 6.5 within 1–3 d during hydroponic growth. Addition of inorganic acids had to be exercised to bring the pH level back to 5.5. Over the 25-d course of hydroponic growth, 12 times of pH regulations were implemented to the HNO_3 treatment and 8 times to the Mixed Acids treatment. For both the H_2SO_4 and H_3PO_4 treatments, only 4 times of acid addition were performed at the beginning of hydroponic growth. Table 1 lists the volumes of acids consumed to regulate the pH level of the nutrient solutions to the optimal range of 5.5–6.5. A total of 1,294 mL of 1 M HNO_3, 573 mL of 1 M H_2SO_4, 880 mL of 1 M H_3PO_4, and 1,080 mL of the mixed acids (648 mL HNO_3, 216 mL H_2SO_4, and 216 mL H_3PO_4) were used.

Changes in pH of the nutrient solutions as a function of hydroponic growth of water spinach were investigated by an intensive, every 2-h monitoring program over 70 h. As shown in Fig. 3(a), all the treatments with the initial pH adjustments to 5.6±0.2 (not Control) exhibited a similar trend of pH shifts within the first 4 h following acid addition: the solution pH increased slowly yet steadily as time progressed. Afterwards, the solution pH of the H_2SO_4 treatment decreased back to 5.5 in another 12 h, rose to 6.1 in the following 6 h, and then decreased gradually to 3.7 at the end of the 3-d monitoring. In comparison, the solution pH of the H_3PO_4 treatment continued to increase to a peak value of 5.9 in 10 h, and then decreased gradually to 3.8 over time. Both the HNO_3 and Mixed Acids treatments, however, raised their solution pH to 6.5 in 20 h and maintained above 6.5 (but below 7.0) to the 64 h. Later the HNO_3 treatment elevated its solution pH continuously to 7.2 at the end of the 72-h monitoring, while the Mixed Acids treatment decreased its solution pH to 6.0.

Fig. 3(b) presents the EC dynamics of the differently treated nutrient solutions as a function of hydroponic growth of water spinach. The pH adjustments did influence the EC of the nutrient solutions; with the H_2SO_4 treatment had the highest EC while the Control had the lowest EC among the five treatments. Nevertheless, all the nutrient solutions maintained the EC in a range of 1200–1400 $\mu S\ cm^{-1}$. The EC fluctuated in the first 66 h. And, then it demonstrated a slight yet clear decreasing trend in the final 4 h of monitoring.

The concentration profiles of NO_3^-, SO_4^{2-}, and water soluble phosphate in the nutrient solutions over the 3-d intensive monitoring course are displayed in Fig. 4. In general, the concentrations of NO_3^- and water soluble phosphate demonstrated a gradually decreasing trend, while the concentration of SO_4^{2-} remained relatively constant. For the HNO_3, Mixed Acids, and Control treatments, the NO_3^- concentration decreased from 618.89 to 71.19 mg L^{-1}, from 191.13 to 4.50 mg L^{-1}, and from139.5 to 0.58 mg L^{-1},

Table 1: Inorganic acids consumed to regulate the pH of the hydroponic nutrient solutions

Treatment	1 M HNO_3 (mL)	1 M H_3PO_4 (mL)	1 M H_2SO_4 (mL)	Total (mL)
Control	0	0	0	0
HNO_3	1294	0	0	1294
H_2SO_4	0	0	573	573
H_3PO_4	0	880	0	880
Mixed Acids	648	216	216	1080

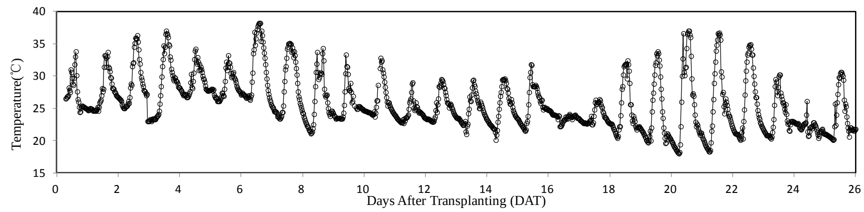

Fig. 1: The 30-min air temperature profile of the hydroponic environment

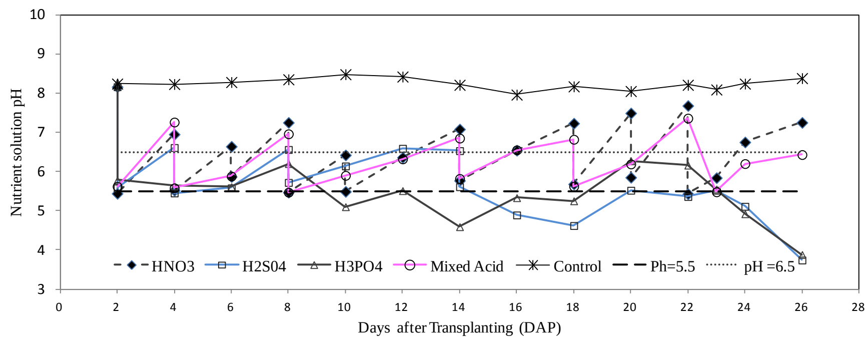

Fig. 2: The pH of nutrient solutions regulated with inorganic acid adjustments every two days during the hydroponic growth of water spinach

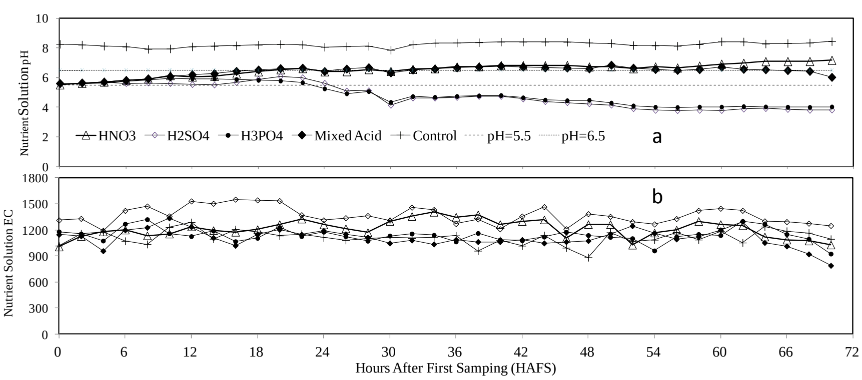

Fig. 3: The 3-d dynamics of nutrient solution pH (a) and electrical conductivity (b) as influenced by hydroponic growth of water spinach

respectively. The nutrient solutions of the H_2SO_4 and H_3PO_4 treatments contained rather low concentrations of NO_3^- (<10 mg L^{-1}) after 21 days of hydroponic growth, approaching 0.5 mg L^{-1} at the end of the 3-d monitoring (Fig. 4a). The concentrations of water soluble phosphate in Control, HNO_3, and H_2SO_4 treatments remained relatively constant around 51, 22, and 48 mg L^{-1}, respectively. For the H_3PO_4 treatment, the nutrient solution decreased its water soluble phosphate concentration from 170 mg L^{-1} to 120 mg L^{-1} in the first 56 h and then elevated it back to approximately 150 mg L^{-1}. For the Mixed Acids treatment, the water soluble phosphate concentration decreased from 82.2 mg L^{-1} to 26.7 mg L^{-1} over the 3-d monitoring course (Fig. 4b). In the same course the average SO_4^{2-} concentrations of the Control, HNO_3, H_2SO_4, H_3PO_4, Mixed Acids treatments maintained relatively constant at 103.7, 117.0, 313.1, 99.8 and 201.7 mg

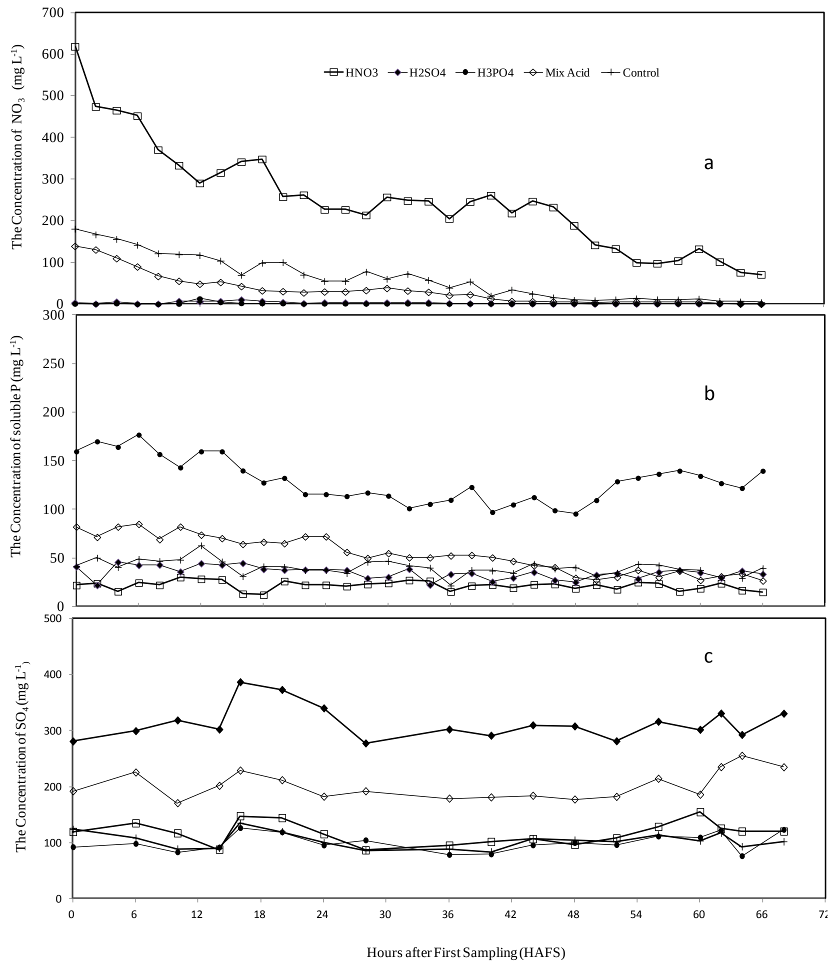

Fig. 4: Changes of concentrations of nitrate (NO_3^-, a), water soluble phosphate (Soluble P, b), and sulfate (SO_4^{2-}, c) in nutrient solution as affected by hydroponic growth of water spinach

L^{-1}, respectively (Fig. 4c).

Yield and Nutritional Quality of Hydroponic Water Spinach

The above-root plant height, fresh weight (FW), dry weight (DW), and FW/DW ratio of water spinach hydroponically grown in the five solution treatments are given in Table 2. Relative to the Control, the HNO_3 and Mixed Acids treatments showed significantly higher plant height, FW, and DW of water spinach, but not in the DW/FW ratio. The H_2SO_4 and H_3PO_4 treatments yielded water spinach similar to or significantly lower plant height, FW and DW yet significantly higher in DW/FW ratios than Control. Among the five treatments, the Mixed Acids treatment followed by the HNO_3 treatment had the highest plant height, FW, and DW of water spinach.

The contents of vitamin C, soluble sugar, crude protein, and NO_3^- in the shoots of water spinach are listed in Table 3. Among the five treatments, the Mixed Acids showed the highest contents of vitamin C (225.3 mg kg^{-1}), soluble sugar (9.32 g kg^{-1}), and crude protein (2.75 mg kg^{-1}). The highest NO_3^- content was observed in the HNO_3 treatment. Compared with the Control, both HNO_3 and Mixed Acids treatments yielded water spinach with significantly higher vitamin C, soluble sugar and crude protein contents. Nevertheless, water spinach from in the both treatments also contained significantly higher contents of NO_3^- (Table 3).

Discussion

The pH value of nutrient solution is an important factor affecting the nutrient availability and plant uptake in soilless culture (Wan *et al.*, 1994; Roosta, 2011). The optimal pH of nutrient solution for hydroponic plant growth is in the range of 5.5–6.5. The pH of the nutrient solution, however, changes with the course of hydroponic growth primarily as a result of plant selective absorption of nutrient ions. It is nearly impossible to maintain the nutrient solution pH in the

Table 2: Plant height and biomass yield of hydroponically grown water spinach. Data are represented as mean ± standard deviation of five replicate measurements

Treatment	Plant height (mm)	Fresh weight (g)	Dry weight (g)	Dry weight/fresh weight (g/g)
Control	41.28±2.46b*	20.6±3.98a*	1.60±0.33a*	0.08±0.021ab*
HNO_3	57.31±5.06c	28.18±2.07b	1.98±0.11ab	0.07±0.007b
H_2SO_4	39.77±2.64b	16.76±1.47a	1.65±0.23a	0.10±0.006a
H_3PO_4	27.72±4.16a	19.59±2.23a	1.79±0.13a	0.10±0.005a
Mixed Acids	68.83±2.48d	36.51±3.12c	2.54±0.18b	0.07±0.007b

*Letters after the numbers in the same column for the same analytical parameter indicate the significance of differences between two treatments by the Duncan test (n = 5) at α = 0.05. Different letters suggest the differences are significant

Table 3: Nutritional quality of water spinach growing in differently treated hydroponic nutrient solutions. Data are represented as mean ± standard deviation of five replicate measurements

Treatment	Vitamin C mg kg^{-1} FW	Nitrate mg kg^{-1} FW	Soluble sugar mg kg^{-1} FW	Crude Protein mg kg^{-1} FW
Control	112.04±17.60b*	1188.45±44.53b*	8.70±0.92bc*	1.75±0.30a*
HNO_3	173.28±20.63c	2236.95±188.25c	6.59±0.72a	2.03±0.03bc
H_2SO_4	91.20±7.19ab	779.43±169.74a	7.74±1.31abc	1.84±0.51a
H_3PO_4	57.79±6.36a	674.83±39.54a	7.13±0.82ab	1.62±0.83a
Mixed Acids	225.30±16.31d	1637.62±267.06bc	9.32±0.59c*	2.75±0.28c

FW: fresh weight

*Letters after the numbers in the same column for the same analytical parameter indicate the significance of differences between two treatments by the Duncan test (n = 3) at α = 0.05. Different letters suggest the differences are significant

optimal range during hydroponic production without external addition of pH buffers. In the present study, inorganic acids were used to adjust the nutrient solution pH from the initially 8.2 and later >6.5 values to 5.5 (Fig. 2). For the HNO_3 treatment in which HNO_3 was used as the pH adjustment, the pH of the nutrient solution increased from 5.5 to 6.5 within 22 h following the acid addition, similar to the results reported by Lykas *et al.* (2006) and Savvas *et al.* (2006). The increase in pH of nutrient solution is mainly related to the release of HCO_3^- and OH^- from plant roots to maintain the charge neutrality in absorbing NO_3^- and other anionic nutrients from nutrient solution (Keltjens and Nijenstein, 1987). The NO_3^- added to the nutrient solution in the form of HNO_3 was absorbed by water spinach, leading to a dramatic rise in pH of the nutrient solution (Fig. 2). When mixed acids were used, a relatively stable pH (5.5–6.5) was achieved during the 3 days of monitoring (Mixed Acids in Fig. 3a), suggesting an equilibrium state of nutrient components in the solution. When single H_3PO_4 or H_2SO_4 was used as the pH adjustment, the nutrient solution pH decreased gradually from 5.5 to 4.2 in 3 days (H_2SO_4 and H_3PO_4 in Fig. 3a). Likely, the leaf vegetable water spinach needed and actually absorbed SO_4^{2-} or water soluble phosphate much less than NO_3^- and therefore, the corresponding HCO_3^- and OH^- released from plant roots could not offset the H^+ released from absorption of cationic nutrients (e.g, Ca^{2+}, Mg^{2+} and K^+), resulting in the pH decreases. The results indicate that single H_3PO_4 or H_2SO_4 is not suitable for buffering the pH of hydroponic nutrient solution; otherwise a harmful low pH environment may be rapidly formed (Islam *et al.*, 1980; Spinu *et al.*, 1997).

The level of pH greatly influences the speciation and nutrient availability such as P, Fe, Mg, Cu and Mn in hydroponic solution. The EC of a nutrient solution is closely related to the sum concentration of dissolved salts in the solution. In the present study, no significant differences in EC were observed among the four nutrient solution treatments other than H_2SO_4, which demonstrated slightly yet consistently higher EC values (Fig. 3). In contrast, the concentrations of NO_3^-, water soluble phosphate, and SO_4^{2-} in the nutrient solutions were significantly different, respectively, among the five treatments (Fig. 4). The decreased concentrations of NO_3^- over time in the HNO_3, Mixed Acids, and Control treatments (Fig. 4a) were attributed to the strong uptake of NO_3^--N by water spinach. The differences of NO_3^- concentration between HNO_3 and Mixed Acids treatments were chiefly caused by the different amounts of HNO_3 added to the hydroponic systems for pH regulation (Table 1). At the same monitoring points, the NO_3^- concentration of Control treatment was clearly higher than that of the Mixed Acids treatment. It might be attributed to the inhibition of plant uptake of NO_3^- from nutrient solution by a high pH environment. Kim *et al.* (2005) reported a similar observation that the NO_3^- uptake by hydroponic rose was restricted by the high pH level (pH=8) of the nutrient solution. In general, leaf crops take much less P and S than N to grow healthy (Brady and Weil, 2007). As a result, the nutrient solutions in the H_3PO_4 and H_2SO_4 treatments maintained relatively high concentrations of water soluble phosphate and SO_4^{2-}, respectively (Fig. 4). Over-supply of water soluble phosphate and SO_4^{2-} may occur, respectively, if H_3PO_4 and H_2SO_4 are used to regulate the pH of hydroponic nutrient solution.

Numerous studies have indicated that the growth of many plants is inhibited in soil and soilless conditions with a high pH level (Bertoni *et al.*, 1992; Zribi and Gharsalli, 2002; Roosta, 2011; Roosta and Rezaei, 2014). It is critical to maintain the pH of the nutrient solution in an appropriate range for best hydroponic production (Voogt and Sonneveld, 2009). In the present study, the fresh weight and dry weight yields of water spinach grown in the H_2SO_4, H_3PO_4 and Control treatments were not significantly different (Table 2), suggesting that single H_2SO_4 or H_3PO_4 used as a pH adjustment of nutrient solution did not promote hydroponic growth of water spinach. A possible reason was that the nutrient solutions shifted to a strongly acidic condition (e.g., pH <4.5, Fig. 2) as a result of the corresponding nutrient uptake by plants. Schubert *et al.* (1990) reported that nutrient uptake of beans was restricted at low pH level (pH=4.0) of growth medium. Yan *et al.* (1992) observed poor growth of corn and broad bean at low growth medium pH (e.g., <4.0). The authors believed that a low pH environment inhibited proton (H^+) release from plant roots and in turn, decreased the cytoplasmic pH. Compared with Control, the HNO_3 and Mixed Acids treatments significantly enhanced the growth of water spinach (Table 2). The highest plant height and fresh/dry weights of water spinach were achieved in the Mixed Acids treatment (Table 2). Mostly, the pH of the nutrient solution in this treatment was in the optimal range of 5.5–6.5 (Fig. 3). Another possible reason was the relatively balanced presence of NO_3^-, SO_4^{2-}, and water soluble phosphate in the nutrient solution from the mixed acid addition (Fig. 4). In comparison, HNO_3 only did not work as effectively as the mixed acids in regulating the solution pH, as indicated by the further shifting of pH away from the 6.5, 22 h after the pH adjustment (Fig. 3).

The vitamin C contents of water spinach showed significant differences among the five nutrient solution treatments, suggesting that the pH adjustments influenced the nutritional quality of hydroponic water spinach. The NO_3^- content of water spinach shoot was positively related to the amount of HNO_3 added to the nutrient solutions (Table 3). Similar results were reported for hydroponically-grown spinach, cabbage and lettuce (Wang and Li, 2004; Liu *et al.*, 2014). However, for all the five treatments, the NO_3^- contents of water spinach did not exceed the maximum limit of 3,000 mg kg^{-1} for leaf vegetables (Shen *et al.*, 1982). The sugar content of water spinach from the Mixed Acids treatment, though not significantly higher than the Control, was the highest among the five treatments (Table 3). A similar phenomenon was observed for the crude protein content of water spinach, indicating that the Mixed Acids treatment also facilitated accumulation of protein in hydroponic plants.

Conclusion

The pH of nutrient solution influenced the growth and nutritional quality of hydroponic crops. When single H_2SO_4 or H_3PO_4 was used to regulate the solution pH to the optimal range of 5.5–6.5, the hydroponic growth of water spinach was significantly inhibited. The inorganic acid HNO_3 as a pH adjustment facilitated the growth of water spinach, but also resulted in significant accumulation of NO_3^- in plant shoots. The mixed acids of 1M HNO_3, H_3PO_4 and H_2SO_4 at 3:1:1 (v/v/v) worked the most effectively as a pH adjustment in enhancing hydroponic plant growth and nutritional quality. The mixed acids were able to maintain the pH of hydroponic nutrient solution in the optimal range and provide a relatively balanced nutrient environment. Mixed HNO_3, H_3PO_4 and H_2SO_4 is recommended to regulate the pH of hydroponic solution. Further research is needed to identify best proportion compositions of HNO_3, H_3PO_4 and H_2SO_4 mixtures as a pH regulator in hydroponic production.

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