Title

Application of alternating current electro-degradation improves retarded growth and quality in lettuce under autotoxicity in successive cultivation

Author(s)

Md. Raihan Talukder, Md. Asaduzzaman, Hideyuki Tanaka, Toshiki Asao

Journal

Scientia Horticulturae Volume 252, 27 June 2019, Pages 324-331

Published

6 April 2019

URL

https://doi.org/10.1016/j.scienta.2019.04.001

Highlights

- Lettuce cultivation in closed hydroponics experiences autotoxicity
- Due to autotoxicity yield and quality decrease in non-renewed nutrient solution
- Yield gradually decrease in accordance to increase culture solution reuse times
- Electro-degradation of culture solution recovers lettuce yield and quality

Application of alternating current electro-degradation improves retarded growth and quality in lettuce under autotoxicity in successive cultivation

Md. Raihan Talukder[a,b,c], Md. Asaduzzaman[a,d], Hideyuki Tanaka[a] and Toshiki Asao[a,*]

[a]Faculty of Life and Environmental Science, Shimane University, 2059 Kamihonjo, Matsue, Shimane 690-1102, Japan.

[b]Faculty of Agriculture, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur 1706, Bangladesh

[c]The United Graduate School of Agricultural Sciences, Tottori University, Koyama-cho, Minami Tottori, Tottori 680-8553, Japan.

[d]Horticulture Research Center, Bangladesh Agricultural Research Institute, Gazipur 1701, Bangladesh.

[*]Corresponding author: T. Asao (E-mail: asao@life.shimane-u.ac.jp).

Abstract

In this study we investigated autotoxicity in lettuce under successive cultivation and applied alternating current electro-degradation (ED) to non-renewed nutrient solution to degrade the accumulated allelochemicals and consequently to improve the retarded yield and quality of lettuce. In our first experiment, lettuce was grown in renewed, non-renewed and non-renewed + ED solution. Every two weeks interval nutrient solution was changed in the renewed treatment while non-renewed solution was unchanged throughout the growing period but major minerals were adjusted bi-weekly. ED was also applied bi-weekly for 24 hours. Compared to renewed solution, shoot fresh weight was decreased to 24% (301.8 g plant^{-1}) in non-renewed solution. But when ED was applied, non-renewed + ED solution produced shoot fresh weight statistically similar to renewed solution plants. In the second experiment, lettuce was grown in renewed nutrient solution, one culture non-renewed (non-renewed 1C), two culture non-renewed (non-renewed 2C) and non-renewed 2C + ED solutions. In non-renewed 1C, the starting solution was fresh nutrient solution while in non-renewed 2C it was once used. Lettuce grown in non-renewed 2C solution produced shoot fresh yield (258.8 g plant^{-1}), about 35% lower compare to renewed solution. When ED was applied to non-renewed 2C solution produced shoot fresh weight 383.3 g $plant^{-1}$ which was statistically similar to renewed solution. Calcium and iron concentration in lettuce followed the similar trend. Therefore, it was evident that yield and quality of lettuce could be improved through ED in non-renewed solution in two successive cultivations using same nutrient solution. In a following study, we tried to determine the proper ED interval in the third experiment. Results showed that ED to non-renewed solution both weekly and bi-weekly equally improved growth, yield and mineral concentration in lettuce. Hence, ED at two weeks interval was sufficient.

Key words: Autotoxicity; electro-degradation; lettuce; successive cultivation; plant factory; closed hydroponics.

1. Introduction

The commercial production of several leafy vegetables in the plant factory with hydroponic system is progressively increased (Goto, 2012; Kozai, 2013; Salisbury and Bugbee, 1988). Stable supply of vegetables with good quality and safety make this production technique more popular. Lettuce (*Lactuca sativa* L.), which has a short growth cycle and high planting density can be produced in large quantity in the plant factory (Seaman, 2015). There are a lot of different hydroponics systems to grow in the plant factory, but one of the most popular methods is with a closed hydroponics system (Takeuchi, 2000; Oka, 2002; Koshikawa and Yasuda, 2003). A closed hydroponics which is frequently known as re-circulating system refers to a hydroponic system in which nutrient solution is not diverted from the system. The nutrient solution flows through the growing medium into a collector where it is recovered, and then it is reused over and over again in the same way. Closed hydroponics system lower water and nutrient consumption, avoids the supply and disposal cost of nutrient solutions and environmentally friendly - minimal potential for localized ground water contamination. Hence, this hydroponic system has been encouraged recently (Ruijs, 1995; Van Os, 1995).

In closed hydroponics, crop production greatly reduced in non-renewed solution. According to Yu and Matsui (1993, 1994), the growth and yield of tomato and cucumber were also reduced in closed hydroponics. Many researchers found this problem in strawberry (Kitazawa et al., 2005), taro (Asao et al., 2003), lettuce (Lee et al., 2006), several leafy vegetables including lettuce (Asao et al., 2004a) and some ornamentals (Asao et al., 2007). Reason behind these reduced growth and yield of crop in closed hydroponics was a problem of autotoxicity. Crop production experiences autotoxicity due to accumulated root exudates in the rhizosphere of culture solution

(Kitazawa et al., 2005; Asao et al., 1998, 2003, 2004a, 2004b, 2007, 2008; Asaduzzaman et al., 2012; Singh et al., 1999; Tang and Young, 1982). The released chemical compounds create problems in closed hydroponic culture systems as they can accumulate and inhibit the growth of the crops. Exposure of these allelochemicals play a massive amount of ecological and physiological roles as they inhibit plant growth (Rice, 1984), alter mineral uptake (Lyu and Blum, 1990; Baziramakenga et al., 1994), disrupt membrane permeability (Baziramakenga et al., 1995), cause stomatal closure and induce water stress (Barkosky and Einhellig, 1993), influence respiration (Penuelas et al., 1996), affect photosynthesis and protein synthesis (Mersie and Singh, 1993; Rohn et al., 2002), impair hormone balance (Holappa and Blum, 1991) and alter enzyme activities (Rohn et al., 2002; Doblinski et al., 2003). Among them, ion uptake and hydraulic conductivity (i.e. water uptake) are worse affected processes since root is the first organ to come into contact with autotoxins in the rhizosphere (Blum et al., 1999). Continuous crop cultivation with recycled nutrient solution, the plant growth was greatly reduced due to presence of the several organic acids in the reused nutrient solution (Kitazawa et al., 2005; Asao et al., 2003, 2004a). In successive cultivation of lettuce using same nutrient solution, the lettuce growth and yield were also reduced by the same phenomenon (Lee et al., 2006). Removal or degradation of phytotoxic substances that have accumulated in the nutrient solution might reduce the crop growth inhibition.

To eliminate these phytotoxic organic acids from the nutrient solution and to mitigate autotoxicity in hydroponic culture, several methods have been tried such as adsorption of allelochemicals by using activated charcoal (Pramanik et al., 2000; Asao et al., 1998, 1999a, 1999b; Yu and Matsui, 1994; Yu et al., 1993), by using amberlite XAD-4 (Lee, 2006) or by degradation of these chemicals using micro-organisms (Asao et al., 2004b). However, the use of activated charcoal creates blocks in the nutrient solution circulation systems in closed hydroponics and it also adsorbed Fe-EDTA from the nutrient solution. Amberlite XAD-4 is expensive and it creates similar problems like activated charcoal. The use of micro-organisms can't recover crop growth and yield completely. Besides these methods, auxin (2, 4-D and NAA) supplementation to plant (Kitazawa et al., 2007), foliar application of amino acids (Mondal et al., 2013, 2015; Talukder et al., 2018) and application of specific LED light to plant (Talukder et al., 2018) can recover plant growth to some extent in spite of having autotoxicity.

Degradation of toxic compounds by electric means is another way to detoxify allelochemicals. Several phenolic compounds, including phenol (Comninellis and Pulgarin, 1991; Fengand Li, 2003; Fleszar and Ploszynka, 1985), catecol (Comninellis and Pulgarin, 1991), and hydroquinone (Comninellisand Pulgarin, 1991; Fleszar and Ploszynka, 1985), in aqueous solutions and even benzene (Fleszar and Ploszynka, 1985) were found to decompose when treated by ED means. These compounds are oxidized rapidly at the anode and decompose to CO_2 (Comninellis and Pulgarin, 1991; Feng and Li, 2003; Fleszar and Ploszynka, 1985).

Based on these findings, benzoic acid from strawberry root exudates has been tried to decompose through direct current electro-degradation (DC-ED) means (Asaduzzaman et al., 2012; Asao et al., 2008). To avoid some awkward issues associated with DC-ED, Talukder et al., (2019) planned to change the power source from DC to alternating current (AC) and found that the application of AC-ED instead of DC-ED in non-renewed solution resulted in degradation of benzoic acid from the closed hydroponics without altering the properties of nutrient solution and confirmed the improved growth, yield and quality of strawberry.

In Japan, lettuce is widely cultivated in greenhouse using the hydroponic systems but, nutrient solutions have not been properly changed, adjusted or analyzed during cultivation. In most circumstances, nutrient solutions are renewed after a single use because of a new start of cultivation, and the used nutrient solution is drained out. However, if the phytotoxic chemicals that accumulate in the nutrient solutions are effectively eliminated, nutrient solution reuse could be more generally adopted in hydroponic cultivation. Therefore, the present study aimed to recover the lettuce growth from autotoxicity by means of an AC-ED machine in the successive lettuce cultivation using same nutrient solution.

2. Materials and methods

2.1. Plant material

Lettuce (*Lectuca sativa* cv. Souther) was used for this experiment. Seeds were (Takii seed company, Japan) sown in a cell trays (48 cm × 24 cm × 4 cm, 72 cells $tray^{-1}$) with vermiculite substrate and were kept in a growth chamber at 25/20 ºC (day/night), 60% relative humidity, fluorescent light with intensity of 140~160 $\mu mol\ m^{-2}\ s^{-1}$ and a 12 hours photoperiod. After 2-3 days seeds were germinated but cell trays were kept there for 14 days after sowing and during this period only fresh water was supplied in the cell trays. After that lettuce seedlings were transferred to the grow beds of hydroponic system in the plastic containers (68 cm × 53 cm × 23 cm) for nursery in an environment control room. The room was maintained at a relative humidity of 60%, temperature 20/20 °C (day/night), CO_2 concentration of 800 ppm, fluorescent light with intensity of 145 μmol m^{-2} s^{-1} and a photoperiod of 12 hours. One hundred seedlings were accommodated in each grow bed and 30 L, 50% standard "Enshi" nutrient solutions were used for each hydroponic system and solution was renewed bi-weekly. Continuous aeration was maintained in the nursery by a pump (Model: MX 808ST-W, Enomoto, Micro Pump Mfg. Co. Ltd., Japan with a maximum flow rate 25 L $min.^{-1}$). Seedlings were kept there for 2 weeks. Then the more homogenous seedlings were selected as planting materials.

2.2. Nutrient solution

Lettuce seedlings were cultured in 50% standard "Enshi" nutrient solution (Hori, 1966). The pH and electrical conductivity of the nutrient solution were 7.15 and 1.4 dS m^{-1}, respectively whereas the electrical conductivity and pH of the tap water used to prepare this nutrient solution were 0.22 dS m^{-1} and 8.18, respectively.

2.3. Electro-degradation of nutrient solution

AC type electrode (designed and built by Yonago Shinko Co., Ltd., Tottori, Japan) was used for ED of benzoic acid or autotoxic chemicals in culture solution used for lettuce. In AC-ED, the electrode had a central core made of titanium with a surface area of 53.1 cm^2 (anode/cathode) which enclosed with cylindrical tube also made of titanium with a surface area of 95.5 cm^2 (cathode/anode) (Talukder et al., 2019). The nutrient solution can pass through the electrode where electro-degradation takes place. The electrodes were coupled with a digital AC power supplier (AD-8735D, AND, Japan). During electro-degradation 500 Hz, 50% duty ratio, 1.8 A and 24 V were maintained. Every time this process was done for 24 hours. Similar electric condition was successfully used to detoxify benzoic acid and other autotoxic chemicals in culture solution of strawberry (Talukder et al., 2019).

2.4. Cultivation of Lettuce

2.4.1. Experiment I

Selected seedlings from the nursery were used as planting material. Control room for lettuce cultivation was maintained by setting the temperature at 20/20 °C (day/night), relative humidity of 60%, CO_2 concentration of 800 ppm, fluorescent light with intensity of 250~280 μmol m^{-2} s^{-1} and a photoperiod of 12 hours. Seedlings were planted to three stage vertical growing beds (125 cm × 90 cm × 10.5 cm). On 6th November 2017, twenty seedlings were planted in each growing bed fixed with urethane cubes (23 mm × 23 mm × 27 mm). Three growing beds were filled with 50% standard "Enshi" nutrient solution with each capacity of 50 L connected to a 300 L reservoir tank. Nutrient solutions were recirculated at 55/5 min. (recirculate/stop) by an automatic pump (KP-101, Koshin, Kyoto, Japan) with an automatic timer (KS-1500, Iuchi, Osaka, Japan) and maximum discharge of 31 L min.$^{-1}$.

There were three types of culture solutions viz. renewed, non-renewed and non-renewed + ED. In case of renewed culture solution, solutions were renewed bi-weekly. While non-renewed nutrient solutions were not replaced by fresh nutrient solution but major nutrients (NO_3^-, PO_4^{3-}, K^+, Ca^{2+} and Fe^{3+}) concentration were adjusted at every two weeks interval as close as possible to the initial concentration of the 50% "Enshi" solution based on the chemical analyses. The ED was applied in the nutrient solution for 24 hours at two weeks interval in the setting as it was described earlier in section 2.3. Small amount of nutrient solution (25 ml) were collected in plastic bottles for the analyses of major nutrients. Nutrient solution was filtered with qualitative filter paper (Advantec Grade no. 131; 125 mm). Major mineral nutrients such as K^+, Ca^{2+}, Mg^{2+} and Fe^{3+} was measured with an atomic absorption spectrophotometer (Z-2000, Hitachi High-Technologies Corporation, Kyoto, Japan), NO_3^- with a compact NO_3^- meter TWIN NO_3^- (B-343, Horiba, Ltd., Japan) and PO_4^{3-} using spectrophotometer at 720 nm (U-2900, Hitachi High Technology, Tokyo, Japan). Finally lettuce plants were harvested after 6 weeks of planting on 18th December 2017. Data were collected on growth attributes and yield of lettuce at the harvest.

2.4.2. Experiment II

Cultivation procedure from planting to harvest and control room conditions were same as experiment I. Planting was done at 4th January 2018 and harvested at 15th February 2018. There were four types of culture solutions viz. (i) renewed, (ii) non-renewed but starting solution was fresh 50% "Enshi" solution simply we called one culture non-renewed solution (Non-renewed 1C) that was similar to non-renewed solution in experiment I, (iii) non-renewed but starting solution was once used culture solution simply we called two culture non-renewed solution (Non-renewed 2C), (iv) Non-renewed 2C+ED. In renewed culture system, nutrient solutions were renewed bi-weekly and in case of non-renewed culture systems, nutrient solutions were not changed throughout the growing period but major mineral nutrients were adjusted bi-weekly like first experiment. The ED process was also same as first experiment.

2.4.3. Experiment III

In third experiment, cultivation procedure and control room conditions were same as experiment I & II. In this experiment, we applied ED at two different intervals. Seedlings were planted on 5 March 2018 and lettuce harvested on 16 April 2018. There were also four types of culture solutions viz. renewed, non-renewed, non-renewed with weekly electro-degradation (non-renewed + weekly ED), non-renewed with bi-weekly electro-degradation (non-renewed + bi-weekly ED). In renewed culture system, nutrient solutions were renewed bi-weekly but in all other treatment, nutrient solutions were not changed throughout the growing period but major mineral nutrients were adjusted bi-weekly like first experiment. ED of nutrient solution was done like previous setting of experiment I & II.

2.5. Determination of mineral concentration in plant parts

Mineral nutrients concentrations in lettuce plants were determined. Plant parts were separated into shoots and roots and kept in a constant temperature oven (DKN812, Yamato Scientific Co. Ltd. Japan) for 72 hours at 80 °C. When the dry matter reaches constant weight, it was ground into powder with a mixer machine (National MX-X53, Japan). Samples weighing 0.25 g were mixed with 8 ml of HNO_3 (60% conc.) and digested by microwave sample preparation system (ETHOS1, Milestone S.r.l, Bergamo, Italy). After digestion samples were measured up to 50 ml of volumetric flask and then filtered with qualitative filter paper (Advantec Grade no. 131, 185 mm). The filtered sample solutions were analyzed for mineral nutrients by atomic absorption spectrophotometer (Z-2310, Hitachi High Technologies Corporation, Tokyo, Japan).

2.6. Measurement of temperature, EC, pH and determination of mineral nutrients of culture solution

Conditions of culture solution such as temperature, EC and pH were recorded at every two weeks interval. EC was measured by EC meter (ES-51, Horiba, Ltd., Kyoto, Japan) while, temperature and pH were measured using pH meter (D-12, Horiba, Ltd., Kyoto, Japan) at each sampling. Amount of mineral nutrient remains in the culture solution were determined following the analytical procedures as described in section 2.4.1. Data were taken three times throughout the growing period.

2.7. Experimental design and statistical analysis

Different types of nutrient solutions were arranged in a completely randomized design with three replications. Analysis of variance for all data was done using computer package MSTAT-

C developed by Russel (1986). The mean differences of each culture solution were separated according to Tukey's test at $P<0.05$.

3. Results

3.1. Effect of ED application in non-renewed solution (Experiment I)

Growth of lettuce significantly decreased in non-renewed nutrient solution compared to renewed solution (Table 1; Fig. 1A). Application of AC-ED in non-renewed solution increased the growth similar to renewed solution. Leaf number, maximum leaf length and width, longest root length, shoot and root dry weights were improved by the ED treatment. Number of leaves was significantly decreased in plants grown in non-renewed solution compared to renewed solution. While application of ED showed statistically similar number of leaves as it was produced in renewed. Likewise, the maximum leaf length and width, longest root length, shoot and root dry weights were significantly reduced in non-renewed solution compared to renewed solution. Due to application of ED to non-renewed solution these growth parameters showed better performance and were statistically similar to renewed solution. Shoot fresh weight was considered as lettuce yield and it was highest (398.3 g plant^{-1}) in renewed culture solution (Fig. 2A) which was statistically similar to non-renewed culture solution with ED (387.2 g plant^{-1}). The lowest shoot fresh weight (301.8 g plant^{-1}) was observed in non-renewed culture solution. ED of non-renewed culture solution significantly affected the mineral nutrient concentration especially calcium and iron in shoot and root of lettuce plants (Table 2). In root and shoot, both calcium and iron concentrations were decreased significantly in non-renewed culture solution. Other minerals like potassium, magnesium and zinc in both plant parts were not significantly affected but average values were relatively low in non-renewed culture solution. Temperature, EC and pH of the culture solutions measured were not differed significantly throughout the growing periods (Table S1). The amounts of minerals (iron, calcium, nitrogen, phosphorus, potassium and magnesium) were also not significantly affected in different culture solutions (Table S1).

3.2. Effect of ED application in two culture non-renewed solution (Experiment II)

The application of ED in the two culture non-renewed solution also significantly influenced the lettuce growth (Table 3; Fig. 1B). All growth parameters such as leaf number, maximum leaf length and width, longest root length, shoot and root dry weights were lowest in the non-renewed 2Csolution plant. Even compare to non-renewed 1C solution, leaf number, leaf length and width, longest root length, shoot and root dry weights were significantly decreased in plants grown in non-renewed 2C solution. In case of renewed solution, plants produced the highest leaf number, maximum leaf length and width, longest root length, shoot and root dry weights. When ED was applied to non-renewed 2C solution, plants showed all these growth parameters statistically similar to renewed culture solution. Shoot fresh weight was highest (400.2 g plant^{-1}) in renewed solution plants (Fig. 2B) and it was lowest (258.8 g plant^{-1}) in non-renewed 2C solution plants. In case of non-renewed 1C solution plants, shoot fresh weight was 310.2 g plant^{-1} and it was statistically higher than that of non-renewed 2C solution. But ED application to non-renewed 2C solution recovered shoot fresh weight and it was observed 383.3 g plant^{-1} which was statistically similar to renewed solution plants.

The calcium and iron in shoot and root of lettuce plants were also significantly influenced by different culture solution but potassium, magnesium and zinc were not affected (Table 4). Calcium and iron concentrations were decreased significantly in non-renewed 1C and non-renewed 2C solution plants both in root and shoot. Calcium and iron concentration in plants grown in renewed solution was significantly higher and was statistically similar to non-renewed 2C solution treated with ED. Potassium, magnesium and zinc concentrations in root and shoot were relatively low in both non-renewed 1C and non-renewed 2C solution. Temperature, EC, pH and minerals (iron, calcium, nitrogen, phosphorus, potassium and magnesium) concentration of the culture solutions measured were also not varied (Table S2) significantly all over the growing periods in different culture solutions.

3.3. Effect of different intervals of ED application in non-renewed solution (Experiment III)

The application of ED in the non-renewed culture solution significantly influenced the lettuce growth but between the two intensities of ED application significant growth differences were not observed (Table 5; Fig. 1C). All growth parameters were significantly affected. In case of renewed solution, plants produced the highest leaf number, maximum leaf length and width, longest root length, shoot and root dry weights whereas these growth parameters were lowest in the non-renewed culture solution. When ED was applied weekly or bi-weekly to the non-renewed solution, plants demonstrated all these growth parameters statistically similar to renewed culture solution. Shoot fresh weight was highest (393.6 g plant^{-1}) in renewed culture solution (Fig. 2C) and it was lowest (280.5 g plant^{-1}) in non-renewed culture solution. But, due to weekly ED application to non-renewed solution, plants produced 377.8 g plant^{-1} shoot fresh weight and it was statistically similar to renewed culture solution. Bi-weekly ED application to non-renewed solution also produced higher shoot fresh weight (382.1 g plant^{-1}) and it was also statistically similar to renewed culture solution and weekly ED application to non-renewed solution.

The calcium and iron concentrations in lettuce plants were also significantly influenced by different culture solution but potassium, magnesium and zinc concentrations were unaffected (Table 6). In root and shoot, calcium and iron concentrations were decreased significantly in plants grown in non-renewed solution. Calcium and iron concentration in plants grown in renewed solution were statistically similar to non-renewed solution treated with ED either weekly or bi-weekly. Temperature, EC, pH and minerals (iron, calcium, nitrogen, phosphorus, potassium and magnesium) concentrations of different culture solution measured were not varied (Table S3) significantly all over the growing periods like experiment I & II.

4. Discussions

In closed hydroponics system, the nutrient solution is recovered, replenished and recycled. This hydroponic technique increases water and nutrient use efficiencies and reduces environmental pollution. Therefore, recent environmental regulations to conserve ground water and to minimize water and fertilizer consumption (Saavas, 2001) have highlighted the importance of nutrient solution recycling and encouraged the shift from open to closed hydroponics.

Closed recycling hydroponic systems have some limitations and accumulation of allelochemicals in the culture solution is one of them. In previous studies, many researchers found allelochemicals in non-renewed solution from root exudation in strawberry (Kitazawa et al., 2005; Asao et al., 2008; Asaduzzaman et al., 2012; Mondal et al., 2013), cucumber (Yu and Matsui, 1994; Asao et al., 1998), several leafy vegetables (Asao et al., 2004a) and some ornamentals plants (Asao et al., 2007) grown in closed hydroponics.

Therefore, the solution has to be eventually renewed. However, the disposal of culture solution is not likely to damage the environment. Lettuce grown in closed hydroponic accumulate many allelochemicals in the culture solutions (Asao et al., 2004a; Lee et al., 2006). For an effective nutrient solution management and consequently, an increase in hydroponic lettuce yield, it is indispensable to alleviate the inhibitory effect of allelochemicals. To maintain culture solution in closed hydroponics free from allelochemicals or below the threshold levels leading to normal growth, we conducted several experiments.

In our first experiment, all growth parameters and yield of lettuce were significantly affected due to the non-renewed nutrient solution (Table1; Fig. 2A). Shoot fresh yield decreased to 24% compared to renewed nutrient solution. The residual minerals concentrations in the culture solution were not varied. Some other important growth factors for hydroponic lettuce culture as described by Furlani et al., (1999) such as EC, pH and temperature of the culture solution also were not varied. Hence, the retarded growth of lettuce in the non-renewed culture solution was mainly due to inhibitory effect of the accumulated allelochemicals.

Allelochemicals delivered into the rhizosphere due to root exudation (Bertin et al., 2003) found responsible for hampering numerous physiological reactions such as transpiration, water utilization, photosystem II (PSII) efficiency, nutrient uptake, dark respiration, ATP synthesis, cell cycle, phyto-hormone metabolism and gene expression, etc. (Inderjit and Duke, 2003; Blum, 2005). That was why; we obtained reduced lettuce growth in non-renewed solution. It is well-known that plants generate more reactive oxygen species (ROS) when exposed to stressful conditions such as accumulation of allelochemicals in the rhizosphere (Yamamoto et al., 2003; Halliwell, 2006; Rhoads et al., 2006). These ROS are either toxic by-products of aerobic metabolism or key regulators of growth, development, and the defense pathway (Mehdy et al., 1996; Laloi et al., 2004; Mittler et al., 2004). Toxic ROS can affect membrane permeability, cause damage to DNA and protein, induce lipid peroxidation and ultimately lead to programmed cell death. Recent findings about the biochemical and physiological effect of natural phyto-toxins have shed light on the rhizosphere interactions (Weir et al., 2004). Several studies have shown that allelochemical stress can cause oxidative damage, as evidenced by enhanced activity of ROS scavenging enzymes and increased degree of membrane lipid peroxidation (Baziramakenga et al., 1995; Politycka, 1996; Yu et al., 2003; Lara-Nunez et al., 2006; Ye et al., 2004, 2006). Furthermore, Bais et al. (2003) found that allelochemicals induce genome-wide changes of gene expression, and ultimately result in the death of the root cells. Therefore, we obtained reduced root growth in non-renewed culture solution. Consequently, damaged roots hamper water and mineral nutrient uptake. As a result, the leaf number plant^{-1}, leaf size, root length, shoot fresh weight, shoot and root dry weight etc. were reduced. Lettuce grown in non-renewed culture solution showed lower mineral concentration especially calcium and iron in their plant parts (Table 2) due to impaired nutrient uptake as a result of accumulation of growth inhibitors in the rhizosphere (Singh et al., 1999). In our previous studies (Talukder et al., 2019; Asaduzzaman et al., 2012), we also observed lowered calcium and iron concentrations in different plant parts of strawberry grown in non-renewed culture solution.

On the other hand, application of ED to the non-renewed culture solution increased the growth, yield and mineral concentrations in lettuce that were similar to renewed culture solution. The possible reason for this improved plant growth performances due to application of ED in non-renewed culture solution might include the degradation of inhibitory chemicals and no negative effects on solution. Similar results were also obtained in closed hydroponic production of strawberry (Talukder et al., 2019; Asaduzzaman et al., 2012).

In second experiment, lettuce was grown in two types of non-renewed solution viz. non-renewed 1C and non-renewed 2C. Growth, yield and minerals concentrations decreased in plants grown in non-renewed 2C solution compared to non-renewed 1C solution and also compared to renewed solution (Table 3 & 4; Fig. 2B). This might be due to higher concentration of allelochemicals in the non-renewed 2C solution. As non-renewed 2C solution was used for longer period for the cultivation of lettuce compare to non-renewed 1C solution, higher amount of allelochemicals accumulated there.

Currently, some research findings detected many organic acids such as benzoic, phenylacetic, cinnamic, p-hydroxybenzoic, lauric, phthalic, vanillic, palmitic, and stearic acids etc. from the root exudates of lettuce grown in non-renewed solution (Asao et al., 2004a; Lee et al., 2006) and identified as major growth inhibitors. Lee et al., (2006) also determined that number and concentration of these organic acids in the nutrient solution highly varied with reuse time, generally showing the increasing trend with the increase reuse time. A few allelochemicals were exuded from the roots at comparatively low concentration in the first culture. Later on numbers of allelochemicals and their concentrations were found increased in the non-renewed 2C solution. As the number of allelochemicals were found increased in the non-renewed 2C solution, they affected plant growth badly by additive or synergistic means (Inderjit, 1996). As a result, more retarded growth of lettuce was obtained in non-renewed 2C solution.

But, while ED was applied to non-renewed 2C solution the growth, yield and minerals (especially calcium and iron) concentration in lettuce significantly increased which were similar to lettuce grown in renewed solution due to the degradation of allelochemicals. Thus, it revealed that two successive cultivation of lettuce with the same nutrient solution could be achieved through electro-degradation of culture solution.

In a following study, we tried to determine the interval of ED application in the third experiment. ED was applied at one week and two weeks interval. Plants grown in non-renewed solution resulted lower growth, yield and minerals concentration resembling first and second culture experiments (Table 5 & 6; Fig. 2C). ED applied both weekly and bi-weekly produced statistically similar growth, yield and minerals concentration in lettuce which were also similar to that of plants grown in renewed culture solution. Therefore, we could decide that bi-weekly ED application was enough for successive lettuce cultivation and additionally, it reduced the electricity cost compare to weekly ED. Recently, several other reports (Talukder et al., 2019; Asaduzzaman et al., 2012) also found different suitable ED application intervals in closed hydroponic for different crops such as tri-weekly ED application for strawberry.

AC-ED machine, a low cost tool, when applied to non-renewed solution in two successive lettuce culture using same nutrients at two week interval completely recovered the retarded lettuce yield from autotoxicity. Total cost of this process was lower than culture solution renewal cost. Moreover, renewal process causes environmental problem due to disposal of used solution. Therefore, use of ED process would be more supportive for lettuce growers.

5. Conclusion

Lettuce cultivation in the non-renewed hydroponics resulted reduced yield and quality. Plants grown in the two culture non-renewed solution resulted more reduced yield and quality of lettuce than one culture non-renewed solution. In the successive cultivation, lettuce grown in the non-renewed solution gradually reduced yield and quality in accordance to the nutrient solution reuse times. Due to ED application to non-renewed solution recovered the retarded yield and quality completely in both one culture non-renewed solution and two culture non-renewed solutions. We suggest that ED treatment to non-renewed solution (300 L) for 24 hours at two week intervals can be applied for complete recovery of the retarded lettuce yield and quality in two or more successive closed hydroponic cultivation using same nutrient solution.

Acknowledgements

We acknowledge the Technology and Development Group, Yonago Shinko Co., Ltd., Tottori, Japan for providing electro-degradation electrodes used for this study.

References

Asaduzzaman, M., Kobayashi, Y., Isogami, K., Tokura, M., Tokumasa, K., Asao, T., 2012. Growth and yield recovery in strawberry plants under autotoxicity through electro-degradation. European J. Hort. Sci. 77, 58-67.

Asao, T., Hasegawa, K., Sueda, Y., Tomita, K., Taniguchi, K., Hosoki, T., Pramanik, M.H.R., 2003. Autotoxicity of root exudates from taro. Scientia Hortic. 97, 389-396.

Asao, T., Kitazawa, H., Ban, T., Pramanik, M.H.R., 2004a. Search of autotoxic substances in some leaf vegetables. J. Jpn. Soc. Hort. Sci. 73, 247-249.

Asao, T., Kitazawa, H., Ban, T., Pramanik, M.H.R., 2008. Electro-degradation of root exudates to mitigate autotoxicity in hydroponically grown strawberry (*Fragaria* × *ananassa* Duch.) plants. HortSci. 43, 2034-2038.

Asao, T., Kitazawa, H., Tomita, K., Suyama, K., Yamamoto, H., Hosoki, T., Pramanik, M.H.R., 2004b. Mitigation of cucumber autotoxicity in hydroponic culture using microbial strain. Scientia Hortic. 99, 207-214.

Asao, T., Kitazawa, H., Ushio, K., Sueda, Y., Ban, T., Pramanik, M.H.R., 2007. Autotoxicity in some ornamentals with means to overcome it. HortSci. 42, 1346-1350.

Asao, T., Ohba, Y., Tomita, K., Ohta, K., Hosoki, T., 1999b. Effects of activated charcoal and dissolved oxygen levels in the hydroponic solution on the growth and yield of cucumber plants. J. Jpn. Soc. Hort. Sci. 68, 1194-1196.

Asao, T., Pramanik, M.H.R., Tomita, K., Ohba, Y., Ohta, K., Hosoki, T., 1999a. Influences of phenolics isolated from the nutrient solution nourishing growing cucumber (*Cucumis sativus* L) plants on fruit yield. J. Jpn. Soc. Hort. Sci. 68, 847-853.

Asao, T., Umeyama, M., Ohta, K., Hosoki, T., Ito, T., Ueda, H., 1998. Decrease of yield of cucumber by non-renewal of the nutrient hydroponic solution and its reversal by supplementation of activated charcoal. J. Jpn. Soc. Hort. Sci. 67, 99-105. [In Japanese with English summary].

Bais, H.P., Vepachedu, R., Gilroy, S., Callaway, R.M., Vivanco, J.M., 2003. Allelopathy and exotic plant invasion: from molecules and genes to species interactions. Sci. 301, 1377-1380.

Barkosky, R.R., Einhellig, F.A., 1993. Effects of salicylic acid on plant-water relationships. J. Chem. Ecol. 19, 237-247.

Baziramakenga, R., Leroux, G.D., Simard, R.R., 1995. Effects of benzoic and cinnamic acids on membrane permeability of soybean roots. J. Chem. Ecol. 21, 1271-1285.

Baziramakenga, R., Simard, R.R., Leroux, G.D., 1994. Effects of benzoic and cinnamic acids on growth, mineral composition and chlorophyll content of soybean roots. J. Chem. Ecol. 20, 2821-2833.

Bertin, C., Yang, X.H., Weston, L.A., 2003. The role of root exudates and allelochemicals in the rhizosphere. Plant and Soil. 256, 67-83.

Blum, U., 2005. Relationships between phenolic acid concentrations, transpiration, water utilization, leaf area expansion, and uptake of phenolic acids: nutrient culture studies. J. Chem. Ecol. 31, 1907-1932.

Blum, U., Shafer, R., Lehmen, M.E., 1999. Evidence for inhibitory allelopathic interactions including phenolic acids in field soils: Concept vs. an experimental model. Crit. Rev. Plant Sci. 18, 673-693.

Comninellis, C.H., Pulgarin, C., 1991. Anodic oxidation of phenol for waste water treatment. J. Appl. Electrochem. 21, 703-708.

Doblinski, P.M.F., Ferrarese, M.L.L., Huber, D.A., Scapim, C.A., Braccini, A.L., Ferrarese, F.O., 2003. Peroxidase and lipid peroxidation of soybean roots in response to p-coumaric and p-hydroxy benzoic acids. Brazilian Archiv. Biol. Tech. 46, 193-198.

Feng, Y.J., Li, X.Y., 2003. Electro-catalytic oxidation of phenol on several metal-oxide electrodes in aqueous solution. Water Res. 37, 2399-407.

Fleszar, B., Ploszynka, J., 1985. An attempt to define benzene and phenol electrochemical oxidation mechanism. Electrochem. Acta. 30, 31-42.

Furlani, P.R., Silveira, L.C.P., Bolonhesi, D., Faquin, V., 1999. Cultivohidropônico de plantas. Campinas: IAC, 52p (BoletimTécnico 180).

Goto, E., 2012. Plant production in a closed plant factory with artificial lighting. Acta Hort. 956, 37-49.

Halliwell, B., 2006. Reactive species and antioxidants. Redox biology is a fundamental theme of aerobic life. Plant Physiology. 141, 312-322.

Holappa, L.D., Blum, U., 1991. Effects of exogenously applied ferulic acid, a potential allelopathic compound, on leaf growth, water utilization, and endogenous abscisic acid levels of tomato, cucumber and beans. J. Chem. Ecol. 17, 865-886.

Hori, Y., 1966. Gravel culture of vegetables and ornamentals. 3. Nutrient solution. Yokendo, Tokyo, Japan. p. 69-80 [In Japanese].

Inderjit, 1996. Plant phenolics in allelopathy. Bot. Rev. 62, 186-202.

Inderjit, Duke, S.O., 2003. Ecophysiological aspect of allelopathy. Planta. 217, 529-539.

Kitazawa, H., Asao, T., Ban, T., Hashimoto, Y., Hosoki, T., 2007. 2,4-D and NAA supplementation mitigates autotoxicity of strawberry in hydroponics. J. Appl. Hort. 9, 26-30.

Kitazawa, H., Asao, T., Ban, T., Pramanik, M.H.R., Hosoki, T., 2005. Autotoxicity of root exudates from strawberry in hydroponic culture. J. Hort. Sci. Biot. 80, 677-680.

Koshikawa, K., Yasuda, M., 2003. Studies on the bench culture with closed hydroponic system in strawberry (Part I). J. Japanese Soc. Hort. Sci. 72, 394 (In Japanese).

Kozai, T., 2013. Plant factory in Japan-current situation and perspectives. Chron. Hortic. 53, 8-11.

Laloi, C., Apel, K., Danon, A., 2004. Reactive oxygen signalling: the latest news. Curr. Opinion Plant Biol. 7, 323-328.

Lara-Nunez, A., Romero-Romero, T., Ventura, J.L., Blancas, V., Anaya, A.L., Cruz-Ortega, R., 2006. Allelochemical stress causes inhibition of growth and oxidative damage in *Lycopersicon esculentum* Mill. Plant Cell Environ. 29, 2009-2016.

Lee, J.G., Lee, B.Y., Lee, H.J., 2006. Accumulation of phytotoxic organic acids in reused nutrient solution during hydroponic cultivation of lettuce (*Lactuca sativa* L.). Scientia Hortic. 110, 119-128.

Lyu, S.W., Blum, U., 1990. Effects of ferulic acid, an allelopathic compound, on net P, K and water uptake by cucumber seedlings in a split-root system. J. Chem. Ecol. 16, 2429-2439.

Mehdy, M.G., Sharma, Y.K., Sathasivan, K., Bays, N.W., 1996. The role of activated oxygen species in plant disease resistance. Physiologia Plantarum. 98, 365-374.

Mersie, W., Singh, M., 1993. Phenolic acids affect photosynthesis and protein synthesis by isolated leaf cells of velvet leaf. J. Chem. Ecol. 19, 1293-1310.

Mittler, R., Vanderauwere, S., Gollery, M., Breusegem, F.V., 2004. Reactive oxygen gene network of plants. Trends Plant Sci. 9, 490-498.

Mondal, F.M., Asaduzzaman, M., Kobayashi, Y., Ban, T., Asao, T., 2013. Recovery from autotoxicity in strawberry by supplementation of amino acids. Scientia Hortic. 164, 137-144.

Mondal, F.M., Asaduzzaman, M., Tanaka, H., Asao, T., 2015. Effects of amino acids on the growth and flowering of *Eustoma grandiflorum* under autotoxicity in closed hydroponic culture. Scientia Hortic. 192, 453-459.

Oka, S., 2002. Development of the labor-saving cultivation techniques by raising the labor-saving cultivars of vegetables (Part I). Bulletin of the National Agriculture Research Centre, Western Region, Okayama Prefecture, 13, 26-27 (In Japanese).

Penuelas, J., Ribas-Carbo, M., Giles, L., 1996. Effects of allelochemicals on plant respiration and oxygen isotope fractionation by the alternative oxyldase. J. Chem. Ecol. 22, 801-805.

Politycka, B., 1996. Peroxidase activity and lipid peroxidation in roots of cucumber seedlings influenced by derivatives of cinnamic and benzoic acids. Acta Physiologiae Plantarum. 18, 365-370.

Pramanik, M.H.R., Nagai, M., Asao, T., Matsui, Y., 2000. Effects of temperature and photoperiod on phytotoxic root exudates of cucumber (*Cucumis sativa*) in hydroponic culture. J. Chem. Ecol. 26, 1953-1967.

Rhoads, D.M., Umbach, A.L., Subbaiah, C.C., Siedow, J.N., 2006. Mitochondrial reactive oxygen species: contribution to oxidative stress and interorganellar signaling. Plant Physiol. 141, 357-366.

Rice, E.L., 1984. Allelopathy. 2nd Ed. Academic Press, Orlando, Florida, 422.

Rohn, S., Rawel, H.M., Kroll, J., 2002. Inhibitory effects of plant phenols on the activity of selected enzymes. J. Agri. Food Chem. 50, 3566-3571.

Ruijs, M.N.A., 1995. Economic evaluation of closed production systems in glasshouse horticulture. Acta Hort. 340, 87-94.

Russel, D.F., 1986. M-STAT Director.Crop and Soil Science Department, Michigan, State University, U.S.A.

Saavas, D., 2001. Nutritional management of Gerbera (*Gerbera jamesonii*) grown in a closed soilless culture system. Acta Hort. 554, 175-182.

Salisbury, F.B., Bugbee, B., 1988. Plant productivity in controlled environments. Symposium on Plant Productivity in Space. HortSci. 23, 293-299.

Seaman, A., 2015. Production guide for organic lettuce. New York State Integrated Pest Management Program, Cornell University (New York State Agricultural Experiment Station, Geneva, NY).

Singh, H.P., Batish, D.R., Kohli, R.K., 1999. Autotoxicity: concept, organisms and ecological significance. Crit. Rev. Plant Sci. 18, 757-772.

Takeuchi, T., 2000. The nourishment uptake of strawberry cultivar 'Akihime' in rockwool hydroponics with a nutrient solution circulating system. Bulletin of Shizuoka Agricultural Experimental Station. 45, 13-23 (In Japanese with English summary).

Talukder, M.R., Asaduzzaman, M., Tanaka, H., Asao, T. 2018. Light-emitting diodes and exogenous amino acids application improve growth and yield of strawberry plants cultivated in recycled hydroponics. Scientia Hortic. 239, 93-103.

Talukder, M.R., Asaduzzaman, M., Tanaka, H., Asao, T., 2019. Electro-degradation of culture solution improves growth, yield and quality of strawberry plants grown in closed hydroponics. Scientia Hortic. 243, 243-251.

Tang, C.S., Young, C.C., 1982. Collection and identification of allelopathic compounds from the undisturbed root system of bitalta limpograss (*Helmarthria altissima*). Plant Physiol. 69, 155-160.

Van Os, E.A., 1995. Engineering and environmental aspects of soilless growing systems. Acta Hort. 396, 25-32.

Weir, T.L., Park, S.W., Vivanco, J.M., 2004. Biochemical and physiological mechanisms mediated by allelochemicals. Curr. Opinion Plant Biol. 7, 472-479.

Yamamoto, Y., Kobayashi, Y., Devi, S.R., Rikiishi, S., Matsumono, H., 2003. Oxidative stress triggered by aluminum in plant roots. Plant Soil. 255, 239-243.

Ye, S.F., Yu, J.Q., Peng, Y.H., Zheng J.H., Zou, L.Y., 2004. Incidence of Fusarium wilt in *Cucumis sativus* L. is promoted by cinnamic acid, an autotoxin in root exudates. Plant Soil. 263, 143-150.

Ye, S.F., Zhou, Y.H., Sun, Y., Zou, L.Y., Yu, J.Q., 2006. Cinnamic acid causes oxidative stress in cucumber roots and promotes incidence of Fusarium wilt. Environ. Exp. Bot. 56, 255-262.

Yu, J.Q., Ye, S.F., Zhang, M.F., Hu, W.H., 2003. Effects of root exudates, aqueous root extracts of cucumber *(Cucumis sativus* L.) and allelochemicals on photosynthesis and antioxidant enzymes in cucumber. Biochem. Syst. Ecol. 31, 129-139.

Yu, J.Q., Lee, K.S., Matsui, Y., 1993. Effect of the addition of activated charcoal to the nutrient solution on the growth of tomato in hydroponic culture. Soil Sci. Plant Nutr. 39, 13-22.

Yu, J.Q., Matsui, Y., 1993. Extraction and identification of phytotoxic substances accumulated in nutrient solution for the hydroponic culture of tomato. Soil Sci. Plant Nutr. 39, 691-700.

Yu, J.Q., Matsui, Y., 1994. Phytotoxic substances in root exudates of cucumber (*Cucumis sativus* L). J. Chem. Ecol. 20, 21-31.

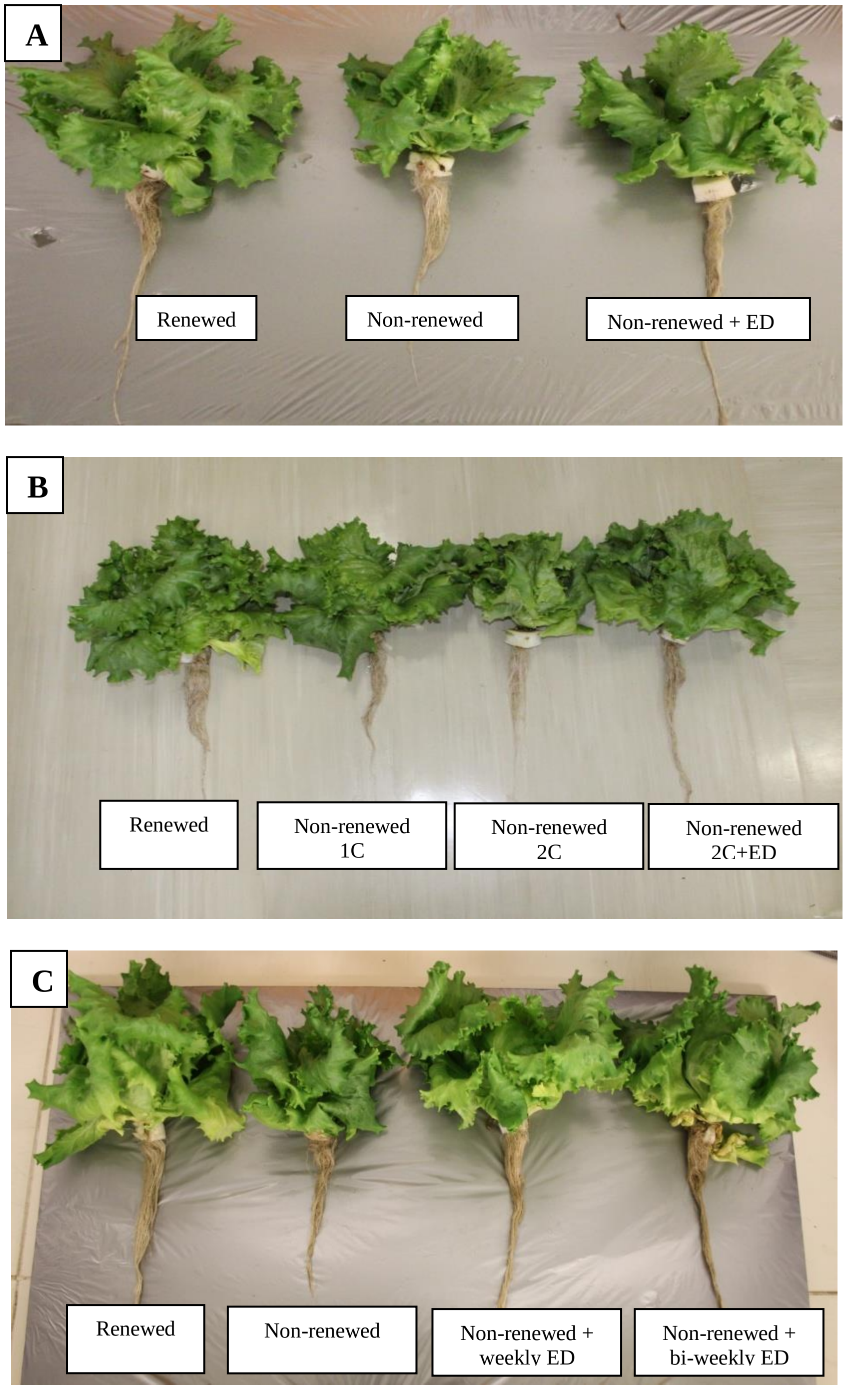

Fig. 1. Effect of electro-degradation (ED) of non-renewed culture solution on the growth of lettuce grown in closed hydroponics, (A) first experiment (B) second experiment and (C) third experiment. ED was applied for 24 hours at every two weeks interval in first and second experiment whereas it was weekly and bi-weekly in third experiment. [Non-renewed 1C = One culture non-renewed solution and Non-renewed 2C = Two culture non-renewed solution].

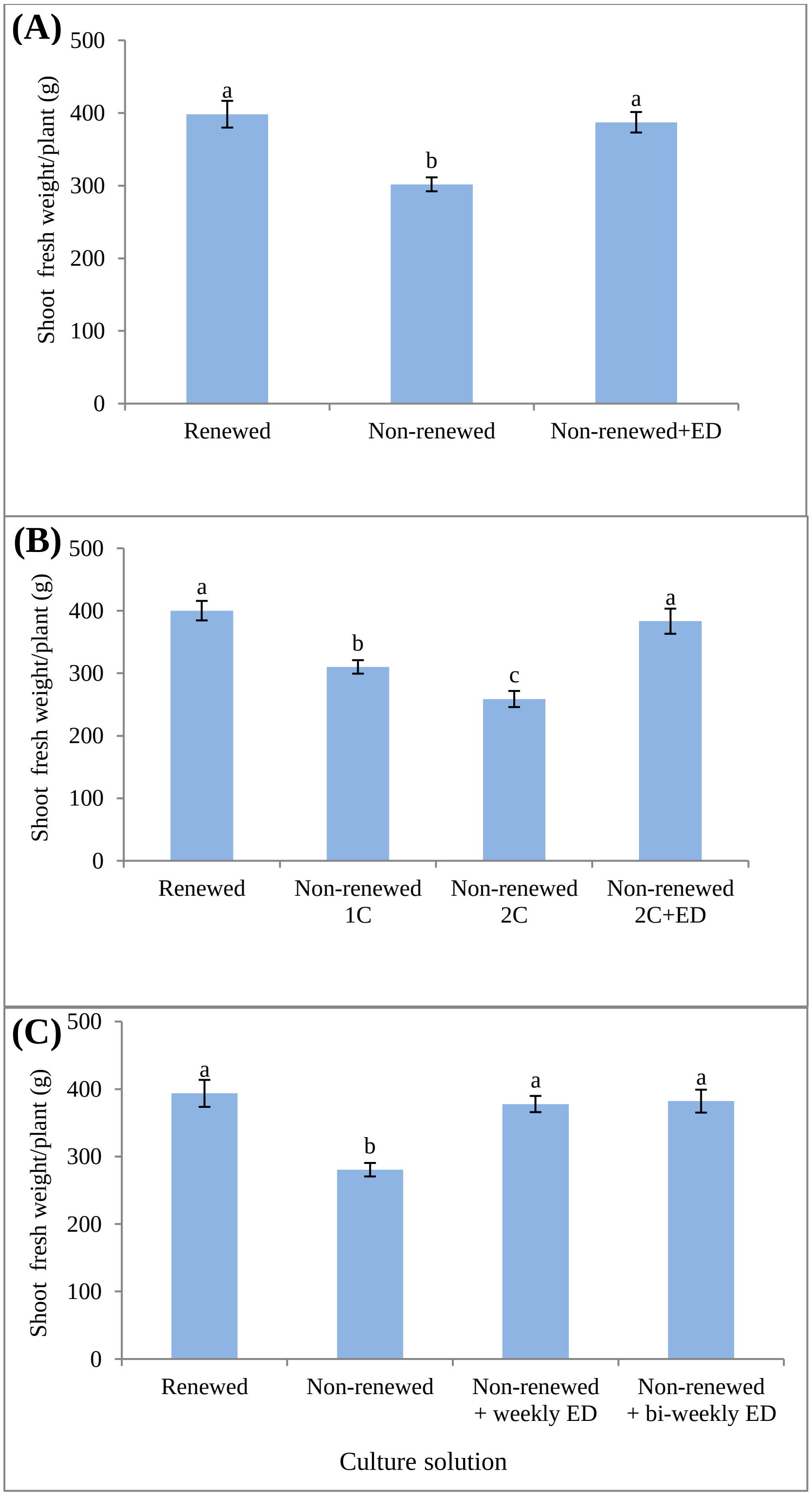

Fig. 2. Effect of electro-degradation (ED) of non-renewed culture solution on the shoot fresh weight of lettuce grown in closed hydroponics, (A) first experiment (B) second experiment and (C) third experiment. ED was applied for 24 hours at every two weeks interval in first and second experiment whereas it was weekly and bi-weekly in third experiment. [Non-renewed 1C = One culture non-renewed solution and Non-renewed 2C = Two culture non-renewed solution].

Table 1. Effect of electro-degradation of non-renewed nutrient solution on the growth of hydroponically grown lettuce plants (Experiment I).

Types of nutrient solution	Number of leaves $plant^{-1}$	Maximum leaf length (cm)	Maximum leaf width (cm)	Longest root length (cm)	Dry weight (g $plant^{-1}$)	
					Shoot	Root
Renewed[z]	22.2 a[w]	32.0 a	26.1 a	50.7 a	10.3 a	1.78 a
Non-renewed[y]	17.7 b	26.5 b	21.3 b	44.9 b	8.5 b	1.29 b
Non-renewed + ED[x]	22.4 a	31.6 a	25.7 a	49.6 a	10.1a	1.72 a

[z]Nutrient solution was renewed bi-weekly.

[y]Nutrient solution was not renewed throughout the culture period but major nutrients were adjusted to standard 50% Enshi solution bi-weekly.

[x]Nutrient solution was not renewed throughout the culture period but major nutrients were adjusted to standard 50% Enshi solution bi-weekly after ED applied.

[w]Means within a column followed by different letters are significantly different according to the Tukey's test at $P<0.05$.

Table 2. Effect of electro-degradation of non-renewed nutrient solution on the mineral concentrations of hydroponically grown lettuce plants (Experiment I).

Types of nutrient solution	Ca (mg g^{-1} DW)		Mg (mg g^{-1} DW)		K (mg g^{-1} DW)		Fe (mg kg^{-1} DW)		Zn ((mg kg^{-1} DW)	
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
Renewed[z]	28a[w]	31 a	3.6	5.1	86	100	496 a	203 a	79	27
Non-renewed[y]	18 b	23 b	3.2	5.0	81	99	314 b	107 b	66	25
Non-renewed + ED[x]	27 a	33 a	3.5	5.2	83	102	511 a	212 a	84	29
Significance			NS	NS	NS	NS			NS	NS

[z]Nutrient solution was renewed bi-weekly.
[y]Nutrient solution was not renewed throughout the culture period but major nutrients were adjusted to standard 50% Enshi solution bi-weekly.
[x]Nutrient solution was not renewed throughout the culture period but major nutrients were adjusted to standard 50% Enshi solution bi-weekly after ED applied.
[w]Means within a column followed by different letters are significantly different and NS indicate non-significant according to the Tukey's test at $P<0.05$.
DW= Dry weight

Table 3. Effect of electro-degradation of two culture non-renewed nutrient solution on the growth of hydroponically grown lettuce plants (Experiment II).

Types of nutrient solution	Number of leaves plant^{-1}	Maximum leaf length (cm)	Maximum leaf width (cm)	Longest root length (cm)	Dry weight (g plant^{-1})	
					Shoot	Root
Renewed[z]	24.2 a[v]	34.3 a	26.7 a	46.3 a	11.0 a	1.85 a
Non-renewed 1C[y]	18.7 b	28.3 b	23.7 b	40.9 b	9.2 bc	1.49 b
Non-renewed 2C[x]	15.1 c	22.7 c	17.0 c	34.3 c	8.4 c	1.06 c
Non-renewed 2C+ED[w]	23.3 a	32.5 a	26.3 a	44.9 ab	10.3 ab	1.67 a

[z]Nutrient solution was renewed bi-weekly.
[y]Nutrient solution was new at starting and was not renewed throughout the culture period but major nutrients were adjusted to standard 50% Enshi solution bi-weekly.
[x]Nutrient solution was one culture used non-renewed solution at starting and was not renewed throughout the culture period but major nutrients were adjusted to standard 50% Enshi solution bi-weekly.
[w]Nutrient solution was one culture used non-renewed solution at starting and was not renewed throughout the culture period but major nutrients were adjusted to standard 50% bi-weekly after ED applied.
[v]Means within a column followed by different letters are significantly different according to the Tukey's test at $P<0.05$.

Table 4. Effect of electro-degradation of two culture non-renewed nutrient solution on the mineral concentrations of hydroponically grown lettuce plants (Experiment II).

Types of nutrient solution	Ca(mg g^{-1} DW)		Mg (mg g^{-1} DW)		K (mg g^{-1} DW)		Fe (mg kg^{-1} DW)		Zn (mg kg^{-1} DW)	
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
Renewed[z]	29a[v]	28 a	3.9	5.0	86	98	510 a	202 a	87	28
Non-renewed 1C[y]	16 b	21 b	3.8	4.8	82	92	344 b	121 b	79	24
Non-renewed 2C[x]	14 b	21 b	3.7	4.7	80	99	312 b	119 b	72	21
Non-renewed 2C+ED[w]	29 a	29 a	3.9	4.9	89	105	558 a	210 a	82	26
Significance			NS	NS	NS	NS			NS	NS

[z]Nutrient solution was renewed bi-weekly.

[y]Nutrient solution was new at starting and was not renewed throughout the culture period but major nutrients were adjusted to standard 50% Enshi solution bi-weekly.

[x]Nutrient solution was one culture used non-renewed solution at starting and was not renewed throughout the culture period but major nutrients were adjusted to standard 50% Enshi solution bi-weekly.

[w]Nutrient solution was one culture used non-renewed solution at starting and was not renewed throughout the culture period but major nutrients were adjusted to standard 50% bi-weekly after ED applied.

[v]Means within a column followed by different letters are significantly different and NS indicate non-significant according to the Tukey's test at $P<0.05$.

DW= Dry weight

Table 5. Effect of different intervals of non- nutrient solution electro-degradation on the growth of hydroponically grown lettuce plants (Experiment III).

Types of nutrient solution	Number of leaves plant^{-1}	Maximum leaf length (cm)	Maximum leaf width (cm)	Longest root length (cm)	Dry weight (g plant^{-1})	
					Shoot	Root
Renewed[z]	23.1 a[v]	33.8 a	27.1 a	42.3 a	10.2 a	1.92 a
Non-renewed[y]	18.7 b	22.3 b	23.3 b	35.9 b	7.7 b	1.28 b
Non-renewed+ED weekly[x]	22.6 a	32.1 a	27.2 a	43.3 a	9.8 a	1.86 a
Non-renewed+ ED Bi-weekly[w]	22.8 a	33.5 a	26.3 a	41.7 a	10.3 a	1.84 a

[z]Nutrient solution was renewed bi-weekly.

[y]Nutrient solution was not renewed throughout the culture period but major nutrients were adjusted to standard 50% Enshi solution bi-weekly

[x]Nutrient solution was not renewed throughout the culture period and major nutrients were adjusted to standard 50% Enshi solution bi-weekly but ED was applied weekly.

[w]Nutrient solution was not renewed throughout the culture period but major nutrients were adjusted to standard 50% Enshi solution bi-weekly and ED was also applied bi-weekly.

[v]Means within a column followed by different letters are significantly different according to the Tukey's test at P<0.05.

Table 6. Effect of different intervals of non- nutrient solution electro-degradation on the mineral concentrations of hydroponically grown lettuce plants (Experiment III).

Types of nutrient solution	Ca(mg g^{-1} DW)		Mg (mg g^{-1} DW)		K (mg g^{-1} DW)		Fe (mg kg^{-1} DW)		Zn (mg kg^{-1} DW))	
	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot	Root	Shoot
Renewed[z]	30a[v]	29 a	4.1	5.4	88	98	560 a	248 a	86	28
Non-renewed[y]	17 b	19 b	3.7	4.9	82	94	313 b	149 b	81	20
Non-renewed+ ED weekly[x]	26 a	27 a	3.9	5.2	86	99	558 a	249 a	82	22
Non-renewed+ ED Bi-weekly[w]	27 a	29 a	3.9	5.3	85	99	626 a	251 a	86	26
Significance			NS	NS	NS	NS			NS	NS

[z]Nutrient solution was renewed bi-weekly.

[y]Nutrient solution was not renewed throughout the culture period but major nutrients were adjusted to standard 50% Enshi solution bi-weekly

[x]Nutrient solution was not renewed throughout the culture period and major nutrients were adjusted to standard 50% Enshi solution bi-weekly but ED was applied weekly.

[w]Nutrient solution was not renewed throughout the culture period but major nutrients were adjusted to standard 50% Enshi solution bi-weekly and ED was also applied bi-weekly.

[v]Means within a column followed by different letters are significantly different and NS indicate non-significant according to the Tukey's test at $P<0.05$.

DW= Dry weight

Table S1.Influence of nutrient solution electro-degradation on the solution temperature, pH, electrical conductivity and residual nutrient concentrations in the experiment I.

Types of nutrient solution	Temperature (ºC)	pH	EC (dS m^{-1})	Residual nutrient content (ppm)					
				Fe^{3+}	Ca^{2+}	Mg^{2+}	K^{+}	NO_3^-	$P_2O_5^-$
Renewed[z]	21.8	7.11	1.36	3.77	104	31.3	79.0	1680	27.3
Non-renewed[y]	21.7	7.11	1.34	3.83	114	31.0	78.3	1663	26.7
Non-renewed + ED[x]	21.1	7.10	1.33	3.74	103	30.3	79.6	1643	26.0
Significance	NS[w]	NS	NS	NS	NS	NS	NS	NS	NS

[z]Nutrient solution was renewed bi-weekly.
[y]Nutrient solution was not renewed throughout the culture period but major nutrients were adjusted to standard 50% Enshi solution bi-weekly.
[x]Nutrient solution was not renewed throughout the culture period but major nutrients were adjusted to standard 50% Enshi solution bi-weekly after ED applied.
[w]NS indicate non-significant according to the Tukey's test at $P<0.05$.

Table S2.Influence of nutrient solution electro-degradation on the solution temperature, pH, electrical conductivity and residual nutrient concentrations in the experiment II.

Types of nutrient solution	Temperature (ºC)	pH	EC (dS m^{-1})	Residual nutrient content (ppm)					
				Fe^{3+}	Ca^{2+}	Mg^{2+}	K^{+}	NO_3^{-}	$P_2O_5^{-}$
Renewed[z]	21.1	7.05	1.32	3.84	101	31.3	77.3	1746	26.0
Non-renewed 1C[y]	21.7	7.10	1.34	3.91	109	31.6	78.3	1732	26.7
Non-renewed 2C[x]	21.0	7.11	1.34	3.86	104	31.0	78.3	1733	26.4
Non-renewed 2C+ED[w]	20.8	7.07	1.34	3.88	103	30.3	79.6	1741	24.6
Significance	NS[v]	NS	NS	NS	NS	NS	NS	NS	NS

[z]Nutrient solution was renewed bi-weekly.
[y]Nutrient solution was new at starting and was not renewed throughout the culture period but major nutrients were adjusted to standard 50% Enshi solution bi-weekly.
[x]Nutrient solution was one culture used non-renewed solution at starting and was not renewed throughout the culture period but major nutrients were adjusted to standard 50% Enshi solution bi-weekly.
[w]Nutrient solution was one culture used non-renewed solution at starting and was not renewed throughout the culture period but major nutrients were adjusted to standard 50% bi-weekly after ED applied.
[v]NS indicate non-significant according to the Tukey's test at $P<0.05$.

Table S3.Influence of nutrient solution electro-degradation on the solution temperature, pH, electrical conductivity and residual nutrient concentrations in the experiment III.

Types of nutrient solution	Temperature (°C)	pH	EC (dS m^{-1})	Residual nutrient content (ppm)					
				Fe^{3+}	Ca^{2+}	Mg^{2+}	K^{+}	NO_3^-	$P_2O_5^-$
Renewed[z]	21.1	7.07	1.29	3.82	105	30.1	77.7	1680	24.7
Non-renewed[y]	21.0	7.08	1.36	3.89	108	31.3	78.3	1780	26.0
Non-renewed+ ED weekly[x]	21.1	7.11	1.34	3.88	104	31.0	78.1	1780	26.7
Non-renewed+ ED Bi-weekly[w]	20.8	7.07	1.34	3.84	103	30.3	79.7	1713	24.6
Significance	NS[v]	NS	NS	NS	NS	NS	NS	NS	NS

[z]Nutrient solution was renewed bi-weekly.
[y]Nutrient solution was not renewed throughout the culture period but major nutrients were adjusted to standard 50% Enshi solution bi-weekly
[x]Nutrient solution was not renewed throughout the culture period and major nutrients were adjusted to standard 50% Enshi solution bi-weekly but ED was applied weekly.
[w]Nutrient solution was not renewed throughout the culture period but major nutrients were adjusted to standard 50% Enshi solution bi-weekly and ED was also applied bi-weekly.
[v]NS indicate non-significant according to the Tukey's test at $P<0.05$.