

# Growth and Quality of Hydroponically Grown Lettuce (*Lactuca sativa* L.) Using Used Nutrient Solution from Coconut-Coir Dust and Hydroton Substrate

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Received 12 February 2016; Accepted 28 April 2016; Available online 15 May 2016

## ABSTRACT

Reuse of used nutrient solution from substrate culture could be increase the water and mineral use efficiency. Therefore, an investigation was conducted to study the effect of used nutrient solution (UNS) on growth and quality of lettuce. Prior to the study, UNS collected from three plant types i.e. green oak leaf lettuce, red coral lettuce and without plant grown in organic substrate coconut-coir dust and inorganic substrate expanded clay granules-hydroton. The nine different combinations of UNS drain and Enshi nutrient solution (ENS) as a control nutrient solution in Deep flow technique for growing green oak leaf lettuce were used as treatments. The results revealed that relative growth reduction was lower in UNS from hydroton compare to UNS from coconut-coir dust. Percent growth reduction of 3.01 to 18.39% and 3.32 to 18.08% in UNS from coconut-coir dust and 1.23 to 13.19% and 0.81 to 12.87% in UNS from hydroton in terms of total fresh and dry matter accumulation respectively, as compared to Enshi nutrient solution (ENS). Among the plant types UNS from red coral lettuce exhibit its better performance across the combination of UNS from substrates. The combination of UNS+ ENS induces influences on the growth and quality of lettuce. The result have an indication that 25% UNS + 75% FNS showed the better performance irrespective of substrate in case of growth and quality traits compared with other treatments.

**KEYWORDS:** Lettuce, used nutrient solution, growth reduction, coconut-coir dust, hydroton

## INTRODUCTION

Lettuce (*Lactuca sativa* L.) is one of the most popular leafy vegetables for fresh consumption around the world, and is considered a good source of health-promoting compounds such as vitamins A, C, calcium, iron, antioxidants like quercetin, caffeic acid and lactupicrin which is anti-carcinogenic [1, 2, 3, 4, 5]. In order to meet the mineral malnutrition and feed the world, protect the environment, improve health and achieve economic growth, a new form of agricultural cultivation is required, soilless culture using for efficient crop production [6]. Lettuce is easily adapted to soilless culture system. Soilless culture is effective growing system for future agriculture when land, water and resources will be limited. Soilless culture offers several advantages over traditional soil culture such as more efficient nutrient regulation, efficient use of water and fertilizers, higher density planting, higher yield per unit area, year round production, higher quality and ease of processing of harvested material on account of minimal contamination from pollutants, pests and pathogens [7, 8, 9]. This system also contribute to sustainable production of vegetables through adoption of most efficient growing

conditions with regard to plant requirements in terms of nutrient elements, water supply, climatic conditions as well as modern managerial practices.

Regarding the adoption of soilless culture for vegetable production in the developing country, the coconut-coir dust culture the most appropriate among the soilless culture technique, because it is cheap, need less equipment and easily to operate and locally available. With increased environmental pressure on green house operations to use sustainable or renewable resources, coconut-coir dust is quickly expanding as the newest environmentally safe substrate [9]. It is an eco-friendly organic growing media for the tropics and more effective for root aeration and nutrient solution management [10]. Coconut-coir dust has many desirable substrate characteristics such as high water holding capacity, excellent drainage, absence of weeds and pathogens, physically resilient, slow decomposition, acceptable pH and cation exchange capacity (CEC), easily wettable, and a renewable resource with no known ecological drawbacks [11, 12, 13, 14, 15, 16, 17, 18]. However, the disadvantage of coconut-coir dust culture is the relatively higher loss of nutrient solution as the drain causing water and nutrient use less effective compared to hydroponics system. The solution to overcome this weakness is to find the way to reuse the used nutrient solution.

The used nutrient solution from growing substrate contains the essential nutrients. Reuse of used nutrient solution for the cultivation of crops could lead to considerable conservation of water resources, plant nutrients use efficiency, and decrease environmental pollution [10, 19]. However, from the literature, the used nutrient solution contains some organic compound consider as toxic for plant growth such as benzoic, phenylacetic, cinnamic, p-hydroxybenzoic, lauric, phthalic, vanillic, palmitic, and stearic acids derived from plant root exudates [20, 21, 22, 23, 24]. Moreover, coconut-coir dust media released toxic compound during decomposition process [25]. Therefore, inside the UNS contains toxic compound derived from plant root exudates and media leachate. Waechter-Kristensen *et al.* (1999) [26] reported that four major sources for the origin of phytotoxic organic compounds in soilless growing systems: incoming water, plant roots, microorganisms and organic growing media. The decrease of toxic extent varies with kinds of media, crop plant, varieties and plant age. To promotes the nutrient use efficiency, economic and environmental advantages the proper management is required to reuse substrate used nutrient solution. Research based information on used nutrient solution from substrate grown leafy vegetable are almost absent. Therefore, the objective of this study is to investigate the growth and quality of lettuce grown in used nutrient solution collected from organic substrate coconut-coir dust and inorganic substrate expanded clay granules-hydroton with special emphasis on physiological parameters associated with growth reduction and to ascertain viable growing media for sustainable production.

## MATERIALS AND METHODS

### *Climatic conditions of the experimental site:*

The experiments were conducted in a greenhouse at the Department of Horticulture, Faculty of Agriculture at Kamphaeng Saen, Kasetsart University, Nakhon Pathom 73140, Thailand (latitude 14°01' N, longitude 99°58' E; 7.460m above sea level) from November 2012 and January 2013. The daily mean temperature and relative humidity were recorded using a data-logger (Hobo-H08-032-08, Onset Computer Corporation, MA, USA). The mean minimum and maximum temperatures in the green house were varied 17.1 to 22.0 °C and 27.7 to 34.5 °C, and relative humidity fluctuated from 48% to 95%, respectively. Photosynthetically active radiation (PAR) was measured using portable light meter (LI-250; LI-COR, Lincoln, NE, USA) and it was varied at plant canopy level between 403 to 836  $\mu\text{mol m}^{-2} \text{S}^{-1}$ .

### *Plant material:*

Seeds of Green oak leaf lettuce (*Lactuca sativa* L., var. *crispa* cv. Kristine RZ, Rijk Zwaan, The Netherlands) were sown in a styrofoam tray (55 cm × 45 cm × 7 cm) within polyurethane sponge as the seedling growing medium. Trays was watered twice daily until wet to ensure healthy seedling germination and growth. Two week-old seedlings (at the first two true leaf stage) were transplanted for hydroponic cultivation.

### *Collection of Used nutrient solution (UNS):*

Prior to the study of effect of used nutrient solution, two separate growing systems were set up by the organic substrate coconut (*Cocos nucifera* L.) coir dust and inorganic substrate expanded clay granules-hydroton with two leafy lettuce cultivar viz. green oak leaf lettuce cv. Kristine RZ and red coral lettuce cv. concord (Rijk Zwaan, The Netherlands) and without plant to collect the UNS. Coconut-coir dust (1.00-2.00 mm) was collected from Thap Sakae District, Prachuap Khiri Khan Province, Thailand and Expanded clay granules-hydroton (3.15-4.00 mm) from Bangkok, Thailand. Each growing system comprised of twelve independent galvanized metal cultivation trays (100 cm × 38 cm × 14 cm) filled with 50 liters of coconut-coir dust and expanded clay granules-hydroton and placed on twelve independent metal trays. Growing media coconut-coir dust and hydroton were washed by the tap water until its electrical conductivity (EC) was reduced below 0.2 mS  $\text{cm}^{-1}$  before placed in cultivation boxes. A plastic supply tank (93 cm × 70 cm × 70 cm) coupled with 200 L

Enshi nutrient solution (ENS) [27], a submersible water pump (WSP-105S, Mitsubishi Electric Automation Co. Ltd., Thailand) and an automatic control unit (Master Clear TS-ET1 492, Rctech, Thailand) regulated the delivery of nutrient solution on a daily basis. Deionized water was used in the preparation of ENS and pH and EC of the ENS were adjusted to 6.0 using 10% HNO<sub>3</sub> as required and 1.2 mS cm<sup>-1</sup>, respectively. Two week-old (at the first two true leaf stage) five green oak leaf lettuce and red coral lettuce seedlings were transplanted into the cultivation trays. Each plant was fertigated for 2 minutes per time, 4 times per day by two drippers with a standard 4.5 L h<sup>-1</sup> discharge at a 1.5 bar working pressure. Each cultivation trays had three pores (6 cm diameter) in bottom side and excess nutrient solution were drained away by polyvinyl chloride (PVC) pipe to catchment plastic pots (25 cm × 25 cm × 27 cm). Nutrient solutions drain were collected for 28 days, on daily basis after transplanting from green oak leaf lettuce, red coral lettuce and without plant grown in coconut-coir dust and hydroton. The collected nutrient solutions drain were stored in individual plastic tanks at the greenhouse for using as used nutrient solution (UNS) in this experiment.

#### *Hydroponic cultivation system:*

Experiments were conducted in deep flow technique (DFT) hydroponic cultivation system. Each experimental system consisted of thirty plastic cultivation trays (41 cm × 28 cm × 14 cm) coupled with 12 L used nutrient solution, styrofoam panel and plastic pot (25 cm × 25 cm × 27 cm). A water pump (Sonic AP 1200 Power Head, LifeTech, Thailand) was used for recirculation of nutrient solution by PVC pipe. Three green oak leaf lettuce seedlings at the two true leaf stage were transplanted into the 1.75 cm thick styrofoam panel. Seedlings roots dipping into continuously aerated nutrient solution to maintain the oxygen availability. The green oak leaf lettuce plants were cultivated for four weeks (28 days) after transplanting.

#### *Nutrient solution:*

The tropical well-known nutrient solution Enshi nutrient solution (ENS) was used as control nutrient solution in this experiment. Full-strength (1 time concentration with 2.4 mS cm<sup>-1</sup>) 1000 ml stock nutrient solution comprised of 950 mg Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O, 810 mg KNO<sub>3</sub>, 500 mg MgSO<sub>4</sub>·7H<sub>2</sub>O and 155 mg NH<sub>4</sub>H<sub>2</sub>PO<sub>4</sub> as macro elements and 23.6 mg Fe-EDTA, 2.86 mg H<sub>3</sub>BO<sub>3</sub>, 2.11 mg MnSO<sub>4</sub>·4H<sub>2</sub>O, 0.22 mg ZnSO<sub>4</sub>·7H<sub>2</sub>O, 0.08 mg CuSO<sub>4</sub>·5H<sub>2</sub>O and 0.025 mg Na<sub>2</sub>MoO<sub>4</sub>·2H<sub>2</sub>O as micro elements. The pH and electrical conductivity (EC) values of the nutrient solutions were regularly measured by means of portable instruments, pH meter (WD-35634-10, pH Testr10, Oakton Instruments, USA) and EC meter (EC Testr11, Oakton Instruments, USA). During the experiment, pH and EC of the UNS and ENS were adjusted to 6.0 using 10% HNO<sub>3</sub> as required and 1.2 mS cm<sup>-1</sup>, respectively.

#### *Treatments and the experimental design:*

Two experiments were conducted in randomized complete block (RCB) design with three replications. One used nutrient solution (UNS) collected from growing three plant types (green oak leaf lettuce, red coral lettuce and without plant) in coconut-coir dust and another UNS collected from growing three plant types (green oak leaf lettuce, red coral lettuce and without plant) in hydroton. Each experimental treatments consisted of nine UNS with different combination of UNS drain and Enshi nutrient solution (ENS) as the follows; UG100, 100% UNS from green oak leaf lettuce; UG50E50, 50% UNS from green oak leaf lettuce + 50% ENS; UG25E75, 25% UNS from green oak leaf lettuce + 75% ENS; UR100, 100% UNS from red coral lettuce; UR50E50, 50% UNS from red coral lettuce + 50% ENS; UR25E75, 25% UNS from red coral lettuce + 75% ENS; UW100, 100% UNS from without plant, UW50E50, 50% UNS from without plant + 50% ENS; UW25E75, 25% UNS from without plant + 75% ENS and E100, Enshi nutrient solution (control nutrient solution).

#### *Growth measurements:*

At harvest, three plants were taken from each plot for measuring growth and physiological parameters, namely, leaf number, canopy width, leaf area, shoot, root and total fresh weight and data were recorded. Shoots and roots of lettuce were dried in an oven at 70 °C for 72 hours before measuring dry weight. The leaf area was measured using an automatic leaf area meter (LI-3100, LI-COR Inc., USA).

#### *Relative leaf chlorophyll content measurement:*

Ten readings per plant were taken on the adaxial surface of fully expanded leaves to determine the leaf relative chlorophyll content in terms of Soil Plant Analysis Development (SPAD) value using a portable chlorophyll meter (SPAD-502; Konica Minolta Sensing Inc., Japan).

#### *Plant tissue analysis:*

The content of ascorbic acid in fresh lettuce leaves (mg<sup>-1</sup>100 g fresh weight) and percentage of N, P, K in oven dried leaves were determined by using the procedure numbers: 45.1.14, 2.4.02, 2.3.02, and 2.5.04, respectively of Association of Official Analytical Chemist methods (AOAC, 1995) [28].

*Statistical analysis:*

The effect of different UNS treatments on the percent reduction of growth was determined as compared to Enshi nutrient solution. Expanded clay granule-hydroton as an inorganic growing media was used to compared with organic growing media coconut-coir dust in view of evaluation of crop growth performance. All the data were subjected to analysis of variance (ANOVA). The mean values were compared by the Duncan's multiple range test (DMRT) at  $P < 0.05$ . The relationships between different parameters were analyzed by the use of linear regression.

**RESULTS AND DISCUSSION***pH and EC values of nutrient solution:*

The mean pH values in used nutrient solution (UNS) and Enshi nutrient solution (ENS) were maintained at 6.0 during the lettuce cultivation in deep flow technique. pH value started to increase in all treatments from 7 days after transplanting. The pH of the UNS and ENS was increased from 6.2 to 7.0 as the growth of lettuce increased (data not shown). Schwarz (1995) [29] reported that an increase in the pH of the nutrient solution during increase plant growth period caused by vigorous uptake of anions by plant. The values of EC in the UNS and ENS were maintained at  $1.2 \text{ mS cm}^{-1}$ . The EC of the UNS and ENS was also increased as the growth of lettuce increased. At the harvesting stage of the lettuce plants, the EC values increased dramatically from 1.4 to  $1.8 \text{ mS cm}^{-1}$ . The high EC values likely occurred because of the high levels of nutrients in the nutrient solution by the additionally supplied nutrients for solution adjustment [10, 19, 24, 30]. The high pH and EC value of the ENS and UNS is needs to adjust daily basis for successful lettuce cultivation.

*Leaf number, canopy width and leaf area:*

Data on number of leaf in used nutrient solution (UNS) from coconut-coir dust and hydroton treatments showed non-significant difference (Table 1). Leaf area in lettuce showed significant difference among the different combinations of UNS treatments from coconut-coir dust and hydroton, even though most treatments did not differ significantly from each other (Table 1). The leaf area of these UNS combinations was significantly increased at 25% UNS + 75% ENS treatment but significantly decreased at UNS 100% treatment, relative to control nutrient solution (100% Enshi nutrient solution) across the UNS from both growing media. Among the lettuce types, the maximum leaf area was recorded in UNS from red coral lettuce while the minimum was recorded in UNS from green oak leaf lettuce. Canopy width also showed similar trend (Table 1).

*Shoot, root and total fresh weight and dry weight:*

Data on shoot, root and total fresh weight and dry matter accumulation in lettuce showed significantly ( $p < 0.05$ ) different among the different combinations of used nutrient solution (UNS) treatment from coconut-coir dust and hydroton, although most treatments did not differ significantly from each other (Table 2 and 3). The total fresh and dry weight was noted 90.41 to 104.15 g and 5.35 to 6.14 g per plant in ENS and UNS treatments from hydroton and 83.85 to 102.74 g and 4.94 to 6.03 g per plant in ENS and UNS treatments from coconut-coir dust, respectively. The growth parameters, shoot and total fresh and dry weight in UNS from coconut-coir dust was significantly ( $p < 0.05$ ) different but most treatments did not differ significantly from each other (Table 2). Root fresh weight and dry weight markedly affected by UNS from coconut-coir dust and the magnitude of decrease varied significantly among the treatments. The lowest root growth in UNS from green oak leaf lettuce. The shoot and total fresh and dry weight in UNS from hydroton was also varied significantly ( $p < 0.05$ ) different but most treatments did not differ significantly from each other (Table 3). UNS collected from coconut-coir dust with without plant growth was relatively lower compared with UNS collected from hydroton with without plant. Among the UNS combinations, 25% UNS + 75% ENS exhibited the better performance across the growing media in case of growth compared with other treatments.

*Percent growth reduction:*

Percent of growth reduction in shoot root, and total fresh and dry matter accumulation of lettuce varied significantly ( $p < 0.01$ ) among the UNS collected from two different growing media as compared with ENS (Table 4 and 5). Relative growth reduction was lower in UNS from hydroton. In contrast, the growth reduction in UNS from coconut-coir dust was markedly affected and showed the higher growth reduction due to plant root exudes and media toxic effect while UNS from hydroton showed only plant root exudes toxic effect [21, 31, 25]. The results exhibited the percent growth reduction of 3.01 to 18.39% and 3.32 to 18.08% in UNS from plant root exudes and media toxic effect (coconut-coir dust) and 1.23 to 13.19% and 0.81 to 12.87% in UNS from plant root exudes toxic effect (hydroton) in terms of total fresh and dry weight respectively, as compared with Enshi nutrient solution. Lee et al. (2006) [24] demonstrated that phytotoxic organic acids such as benzoic, phenylacetic, phthalic, palmitic, cinnamic, lauric, and stearic acids were accumulated in reused nutrient solution by root exudes and were to inhibit lettuce growth.

The magnitude of decrease significantly ( $p < 0.01$ ) varied among the UNS collected from two lettuce types and without plant (Table 4 and 5). UNS from red coral lettuce demonstrated the minimum percent growth reduction and maximum percent growth reduction was found in UNS from green oak leaf lettuce. Lettuce grown in UNS collected from coconut-coir dust with without plant growth reduction was relatively higher compared with UNS collected from hydroton with without plant. UNS collected from coconut-coir dust with without plant affected by growing media toxic effect [25] and UNS collected from hydroton with without plant had no media toxic effect because of hydroton is stable growing media [32]. Among the plant types UNS from red coral lettuce exhibit its better performance across the combination of UNS from coconut-coir dust and hydroton. Asao *et al.*, 2001 and 2004 [33, 23] also elucidated plant types differences in the autotoxicity and the identification of phytotoxic organic acids in leafy vegetables including lettuce.

Percent root, shoot and total fresh and dry matter accumulation reduction under different combinations of UNS relative to respective control nutrient solution, significantly ( $p < 0.01$ ) varied among the treatments (Table 4 and 5). The combination of UNS+ ENS induces influences on the performance of crops. Among the UNS combination, 25% UNS + 75% ENS exhibited the better performance across the UNS from growing media in case of growth compared with other treatments. The result has an indication that higher doses of UNS from coconut-coir dust and hydroton showed poor performance on crop growth. The results of this study agree with the findings of Park *et al.* (2005) [34] who reported combined treatment with chemical fertilizer (70%) and waste nutrient solution (30%) promoted the crop growth and yield of red pepper (*Capsicum annum* L.).

Roots are the main plant organs that are direct contact with nutrient solution in hydroponics; therefore, the application of nutrient solution has a direct effect on the growth of crops [35]. The root growth reduction of lettuce decreased significantly ( $p < 0.01$ ) with increasing UNS as compared with control nutrient solution (Table 4 and 5). The results indicated that percent growth reduction was 3.47 to 21.35% and 3.92 to 20.59% in UNS from coconut-coir dust and 1.35 to 15.56% and 1.89 to 16.04% in UNS from hydroton in terms of root fresh and dry weight respectively, as compared with Enshi nutrient solution. The least (4.59%) growth reduction was observed in UNS from hydroton with red coral lettuce and 25% UNS + 75% ENS combination treatment. The maximum (21.35%) growth reduction was occurred in UNS from coconut-coir dust with green oak lettuce and 100% UNS combination treatment. Vaughan and Ord (1990) [36] found that phenolic acids such as ferulic, vanillic, p-hydroxybenzoic, syringic, and caffeic acid inhibited growth of roots of pea cultivated in Hoagland nutrient solution under axenic conditions. Ma and Nichols (2004) [25] also demonstrated that the phytotoxicity was attributed to the phenolic compounds in the coconut-coir substrates and were severely inhibited root growth of lettuce.

#### *Mineral elements in lettuce:*

Data on percent of mineral elements in lettuce like phosphorus and potassium showed non-significant difference (Table 6). Percent of nitrogen in lettuce leaf showed significant difference among the different combinations of UNS treatments from coconut-coir dust and hydroton, while most treatments did not differ significantly from each other (Table 6). The percent of nitrogen of these UNS combinations was significantly increased at 25% UNS + 75% ENS but significantly decreased at UNS 100%, relative to control nutrient solution (100% ENS) across the UNS from both growing media (Table 6). Resh (2013) [9] reported that percent of N, P and K content in lettuce leaf varied 3.0 to 6.0, 0.80 to 1.30 and 5.0 to 10.8, respectively.

#### *Relative leaf chlorophyll content and Ascorbic acid:*

Data on relative leaf chlorophyll content in terms of Soil and Plant Analysis Development (SPAD) value and ascorbic acid in lettuce showed significant difference among the different combinations of UNS treatment from coconut-coir dust and hydroton (Fig 1 and 2). The relative leaf chlorophyll content (SPAD value) and ascorbic acid of these UNS combinations was significantly increased at 25% UNS + 75% ENS but significantly decreased at UNS 100% relative to Enshi nutrient solution across the UNS from both growing media. Among the plant types, the highest relative leaf chlorophyll content and ascorbic acid was recorded in UNS from red coral lettuce while the lowest was recorded in UNS from green oak leaf lettuce. The results indicated that, higher UNS combinations reduced leaf chlorophyll and ascorbic acid content but the increase in chlorophyll and ascorbic acid content observed at 25% UNS + 75% ENS treatment and red coral lettuce relative to ENS [10]. Relative leaf chlorophyll content in leaf tissue which are key for photosynthetic performance and thereby attained enhanced the crop growth. Moreover, nitrogen is one of the most important mineral nutrients determining plant growth and crop yield. Its effects are associated with photosynthetic rate. The increases of relative leaf chlorophyll content in lettuce due to the increases nitrogen. Therefore, the increase in chlorophyll content at 25% UNS + 75% ENS relative to 100% ENS might be due to such a mechanism [10]. In contrast, the chlorophyll content decrease under high (100% UNS) concentration of UNS relative to control indicated possible inhibition of nitrogen synthesis.

**Table 1:** Leaf number, canopy width and leaf area of Green oak leaf lettuce grown in hydroponically using used nutrient solution (UNS) from three plant types previously grown in coconut- coir dust and hydroton

Treatments	UNS from Coconut-coir dust			UNS from Hydroton		
	Leaf no. plant <sup>-1</sup>	Canopy width (cm)	Leaf area (cm <sup>2</sup> plant <sup>-1</sup> )	Leaf no. plant <sup>-1</sup>	Canopy width (cm)	Leaf area (cm <sup>2</sup> plant <sup>-1</sup> )
UG100	18.22	20.64d	1088.44e	19.66	22.11d	1179.28e
UG50E50	19.67	22.02cd	1172.21de	20.50	23.17c	1271.91d
UG25E75	20.75	23.27b-d	1244.73dc	21.15	25.52ab	1380.56c
UR100	19.50	21.61cd	1139.97e	20.33	23.27c	1258.91de
UR50E50	20.83	23.12b-d	1251.19dc	22.83	24.35bc	1373.78c
UR25E75	21.50	24.39bc	1308.16bc	23.25	26.29ab	1454.15a-c
UW100	20.67	23.14b-d	1269.53bc	22.75	25.46ab	1395.93bc
UW50E50	21.33	24.19bc	1318.60bc	23.15	25.74ab	1432.20bc
UW25E75	22.67	25.05ab	1357.73ab	23.50	26.81a	1483.12ab
E100	23.75	27.23a	1439.68a	24.25	27.53a	1526.14a
F-test	NS	*	**	NS	*	**
CV (%)	12.48	6.35	4.09	16.51	4.62	3.57

Means followed by common letters are not significant difference as determined by Duncan's New Multiple Range Test (DMRT) at the  $P < 0.05$ ; \* significant at  $P < 0.05$ ; \*\* significant at  $P < 0.01$ ; NS= non-significant difference at  $P < 0.05$ .

UG100 = 100% UNS from green oak leaf lettuce; UG50E50 = 50% UNS from green oak leaf lettuce + 50% ENS; UG25E75 = 25% UNS from green oak leaf lettuce + 75% ENS; UR100 = 100% UNS from red coral lettuce; UR50E50 = 50% UNS from red coral lettuce + 50% ENS; UR25E75 = 25% UNS from red coral lettuce + 75% ENS; UW100 = 100% UNS from without-plant; UW50E50 = 50% UNS from without-plant + 50% ENS; UW25E75 = 25% UNS from without-plant + 75% ENS and E100 = Enshi nutrient solution (control nutrient solution)

**Table 2:** Shoot, root and total fresh weight and dry weight of Green oak leaf lettuce grown in hydroponically using used nutrient solution (UNS) from three plant types previously grown in coconut- coir dust

Treatments	Fresh weight (g plant <sup>-1</sup> )			Dry weight (g plant <sup>-1</sup> )		
	Shoot	Root	Total	Shoot	Root	Total
UG100	75.23d	8.62f	83.85d	4.13d	0.81f	4.94d
UG50E50	80.98b-d	9.21d-f	90.19b-d	4.41b-d	0.87ed	5.28b-d
UG25E75	84.51a-c	9.83b-d	94.34a-c	4.59a-c	0.92b-d	5.51a-d
UR100	78.47dc	8.85ef	87.32dc	4.32dc	0.83ef	5.15dc
UR50E50	82.69b-d	9.49c-e	92.18b-d	4.49b-d	0.90cd	5.39b-d
UR25E75	86.30a-c	10.16bc	96.46a-c	4.68a-c	0.94bc	5.62a-c
UW100	85.12a-c	10.05bc	95.17a-c	4.63a-c	0.93b-d	5.56a-c
UW50E50	87.10ab	10.33ab	97.43a-c	4.75a-c	0.96bc	5.71a-c
UW25E75	89.07ab	10.58ab	99.65ab	4.85ab	0.98ab	5.83ab
E100	91.78a	10.96a	102.74a	5.01a	1.02a	6.03a
F-test	*	**	*	*	**	*
CV (%)	5.14	4.20	5.78	5.08	3.58	5.87

Means followed by common letters are not significant difference as determined by Duncan's New Multiple Range Test (DMRT) at the  $P < 0.05$ ; \* significant at  $P < 0.05$  and \*\* significant at  $P < 0.01$

Treatment abbreviations as in Table 1.

**Table 3:** Shoot, root and total fresh weight and dry weight of Green oak leaf lettuce grown in hydroponically using used nutrient solution (UNS) from three plant types previously grown in hydroton

Treatments	Fresh weight (g plant <sup>-1</sup> )			Dry weight (g plant <sup>-1</sup> )		
	Shoot	Root	Total	Shoot	Root	Total
UG100	81.02d	9.39c	90.41c	4.46c	0.89c	5.35c
UG50E50	85.33b-d	9.92bc	95.25a-c	4.67a-c	0.94bc	5.61a-c
UG25E75	88.37a-c	10.33a-c	98.70a-c	4.88ab	0.97a-c	5.85a-c
UR100	82.84dc	9.58c	92.42bc	4.57bc	0.91c	5.48bc
UR50E50	87.32a-c	10.21a-c	97.53a-c	4.80a-c	0.96a-c	5.76a-c
UR25E75	89.23ab	10.61ab	99.84ab	4.93ab	0.99a-c	5.92ab
UW100	90.04ab	10.75ab	100.79ab	4.95ab	1.02ab	5.97ab
UW50E50	90.82ab	10.86ab	101.68a	5.01a	1.03ab	6.04a
UW25E75	91.90a	10.97a	102.87a	5.05a	1.04ab	6.09a
E100	93.03a	11.12a	104.15a	5.08a	1.06a	6.14a
F-test	*	*	*	*	*	*
CV (%)	3.76	5.19	4.64	4.41	5.35	4.78

Means followed by common letters are not significant difference as determined by Duncan's New Multiple Range Test (DMRT) at the  $P < 0.05$ ; \* significant at  $P < 0.05$  and \*\* significant at  $P < 0.01$

Treatment abbreviations as in Table 1.

**Table 4:** Percent growth reduction of Green oak leaf lettuce grown in hydroponically using used nutrient solution (UNS) from three plant types previously grown in coconut-coir dust as compared with using fresh Enshi nutrient solution

Treatments	% growth reduction compared with ENS					
	Fresh weight			Dry weight		
	Shoot	Root	Total	Shoot	Root	Total
UG100	18.03a	21.35a	18.39a	17.56a	20.59a	18.08a
UG50E50	11.77c	15.97c	12.22c	11.98c	14.71c	12.44c
UG25E75	7.92e	10.31e	8.18e	8.38e	9.80e	8.62e
UR100	14.50b	19.25b	15.01b	13.77b	18.63b	14.59b
UR50E50	9.90d	13.41d	10.28d	10.38d	11.76d	10.61d
UR25E75	5.97f	7.30g	6.11g	6.59g	7.84g	6.80g
UW100	7.26e	8.30f	7.37f	7.58f	8.82f	7.79f
UW50E50	5.10g	5.75h	5.17h	5.19h	5.88h	5.31h
UW25E75	2.95h	3.47i	3.01i	3.19i	3.92i	3.32i
E100	-	-	-	-	-	-
F-test	**	**	**	**	**	**
CV (%)	5.22	4.85	4.65	4.90	4.97	4.88

Treatment abbreviations as in Table 1.

Means followed by common letters are not significant difference as determined by Duncan's New Multiple Range Test (DMRT) at the  $P < 0.01$  \*\* significant at  $P < 0.01$

**Table 5:** Percent growth reduction of Green oak leaf lettuce grown in hydroponically using used nutrient solution (UNS) from three plant types previously grown in hydroton as compared with using fresh Enshi nutrient solution

Treatment	% growth reduction compared with ENS					
	Fresh weight			Dry weight		
	Shoot	Root	Total	Shoot	Root	Total
UG100	12.91a	15.56a	13.19a	12.20a	16.04a	12.87a
UG50E50	8.28c	10.79c	8.55c	8.07c	11.32c	8.63c
UG25E75	5.01e	7.10e	5.23e	3.94e	8.49e	4.72e
UR100	10.95b	13.85b	11.26b	10.04b	14.15b	10.75b
UR50E50	6.14d	8.18d	6.36d	5.51d	9.43d	6.19d
UR25E75	4.08f	4.59f	4.14f	2.95f	6.60f	3.58f
UW100	3.21g	3.33g	3.23g	2.56f	3.77g	2.77g
UW50E50	2.38h	2.34h	2.37h	1.38g	2.83h	1.63h
UW25E75	1.21i	1.35i	1.23i	0.59h	1.89i	0.81i
E100	-	-	-	-	-	-
F-test	**	**	**	**	**	**
CV (%)	6.18	6.77	5.93	8.35	6.41	8.07

Treatment abbreviations as in Table 1.

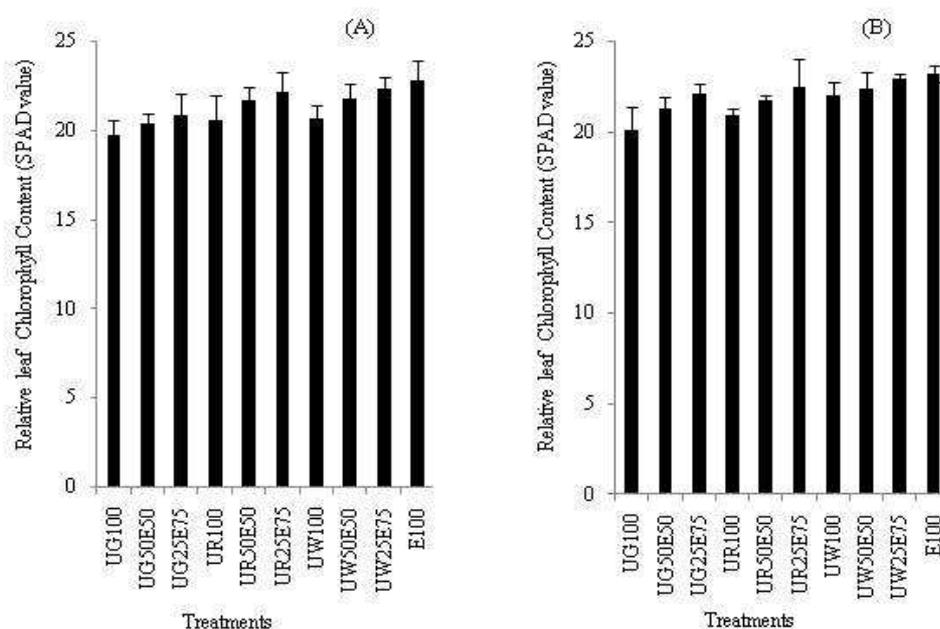
Means followed by common letters are not significant difference as determined by Duncan's New Multiple Range Test (DMRT) at the  $P < 0.01$  \*\* significant at  $P < 0.01$

**Table 6:** Percent N, P, and K content in Green oak leaf lettuce grown in hydroponically using used nutrient solution (UNS) from three plant type previously grown in coconut-coir dust and hydroton

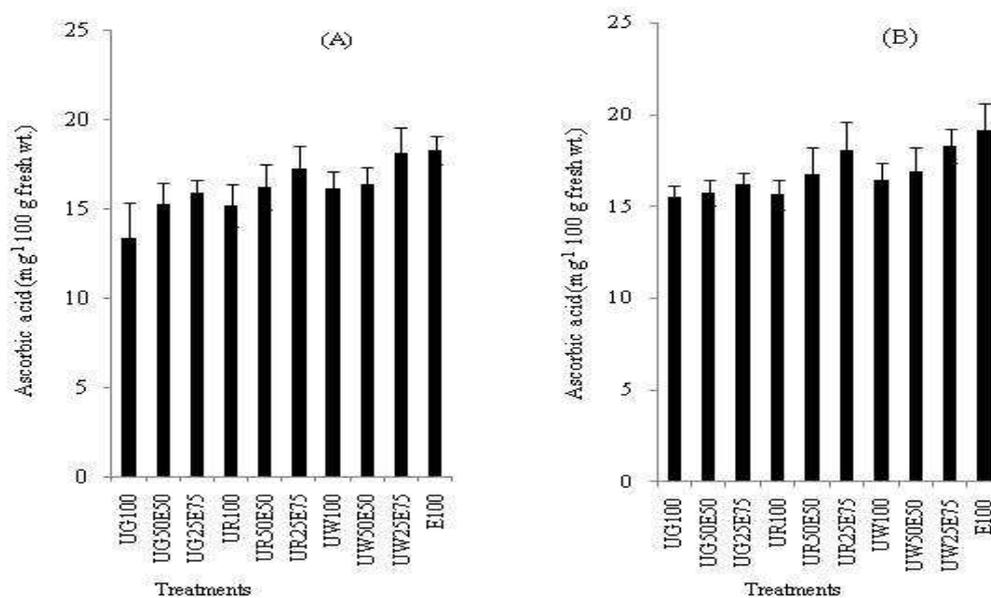
Treatment	UNS from Coconut-coir dust			UNS from Hydroton		
	N	P	K	N	P	K
	(%)					
UG100	3.95d	0.78	3.49	4.45c	0.94	3.04
UG50E50	4.19dc	0.81	3.68	4.59c	0.97	3.11
UG25E75	4.41bc	0.88	3.81	4.73bc	1.08	3.22
UR100	4.17dc	0.92	3.74	4.61c	1.01	3.27
UR50E50	4.39bc	0.95	3.86	5.07ab	1.05	3.39
UR25E75	4.51bc	1.01	4.17	5.26a	1.15	3.77
UW100	4.57bc	0.96	4.02	5.04ab	1.11	3.72
UW50E50	4.66b	0.98	4.15	5.15a	1.12	3.98
UW25E75	4.71b	1.01	4.31	5.18a	1.15	4.13
E100	5.11a	1.14	4.37	5.24a	1.21	4.29
F-test	*	NS	NS	*	NS	NS
CV (%)	5.08	14.97	13.95	4.16	13.53	14.42

Treatment abbreviations as in Table 1.

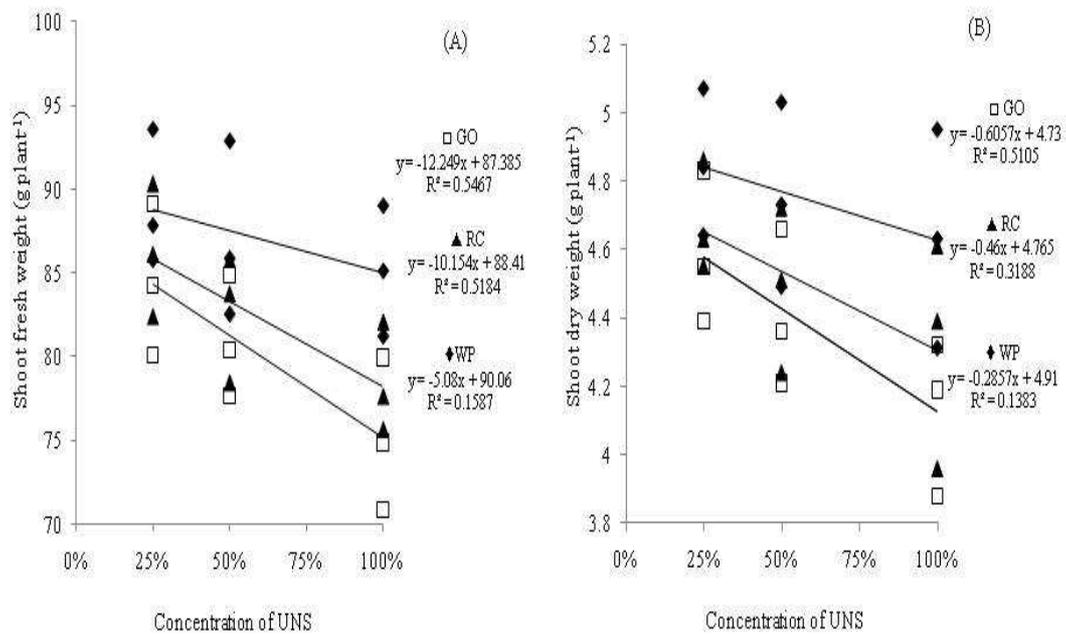
Means followed by common letters are not significant difference as determined by Duncan's New Multiple Range Test (DMRT) at the  $P < 0.05$  \* significant at  $P < 0.05$ ; NS= non-significant difference at  $P < 0.05$ .



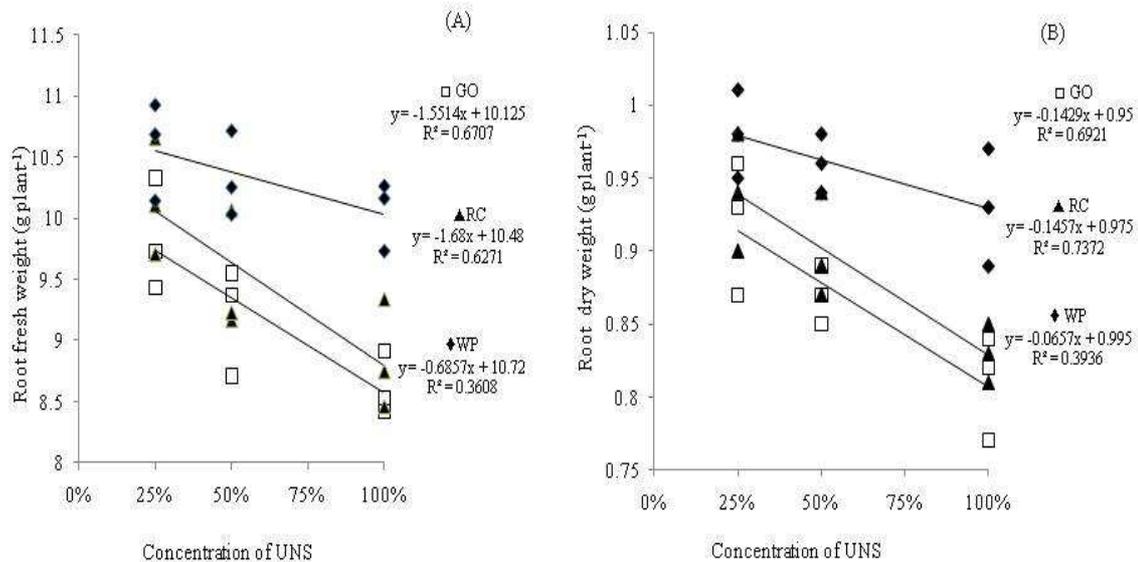
**Fig. 1:** Relative leaf chlorophyll content (SPAD value) of Green oak leaf lettuce grown in hydroponically using used nutrient solution (UNS) from previously grown three plant types in coconut-coir dust (A) and hydroton (B) (vertical error bars =  $\pm$ SD).



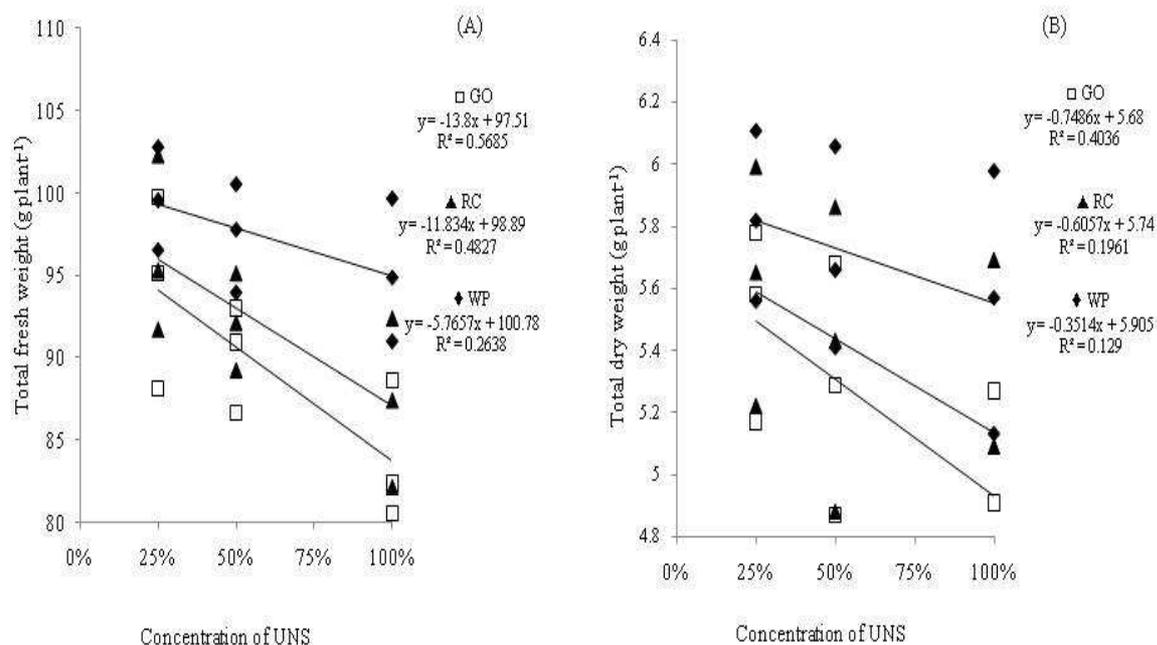
**Fig. 2:** Ascorbic acid content of Green oak leaf lettuce grown in hydroponically using used nutrient solution (UNS) from previously grown three plant types in coconut-coir dust (A) and hydroton (B) (vertical error bars =  $\pm$ SD).



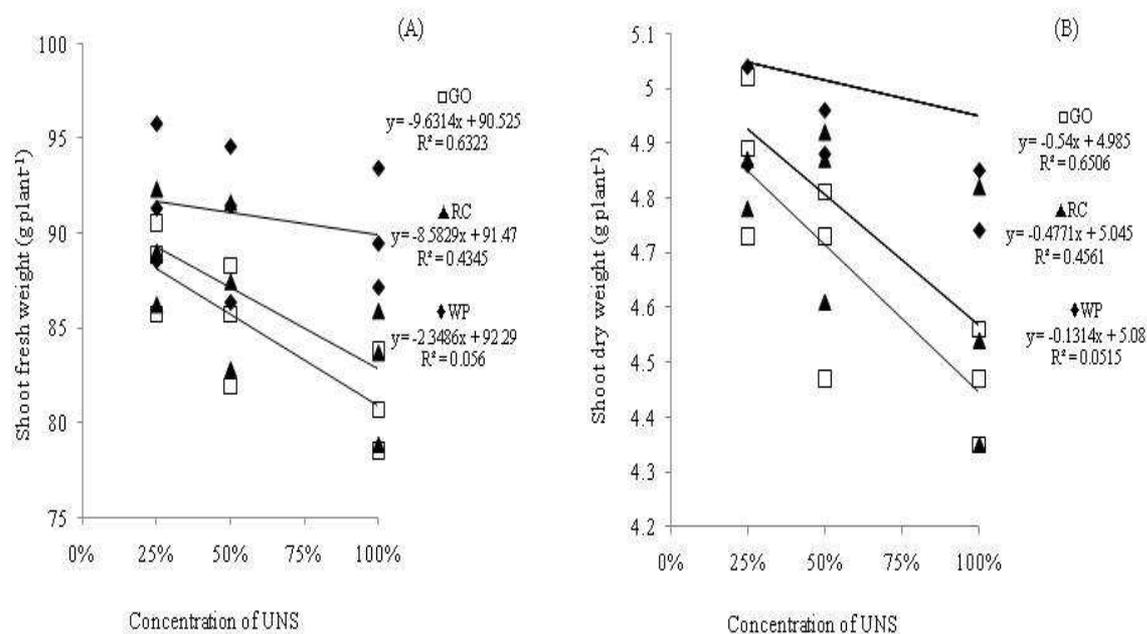
**Fig. 3:** Relationship between: (A) Shoot fresh weight; (B) Shoot dry weight of Green oak leaf lettuce grown in hydroponically and percentage of used nutrient solution (UNS) in nutrient solution derived from previously grown green oak leaf lettuce (GO), red coral lettuce (RC) and without plant (WP) in coconut-coir dust



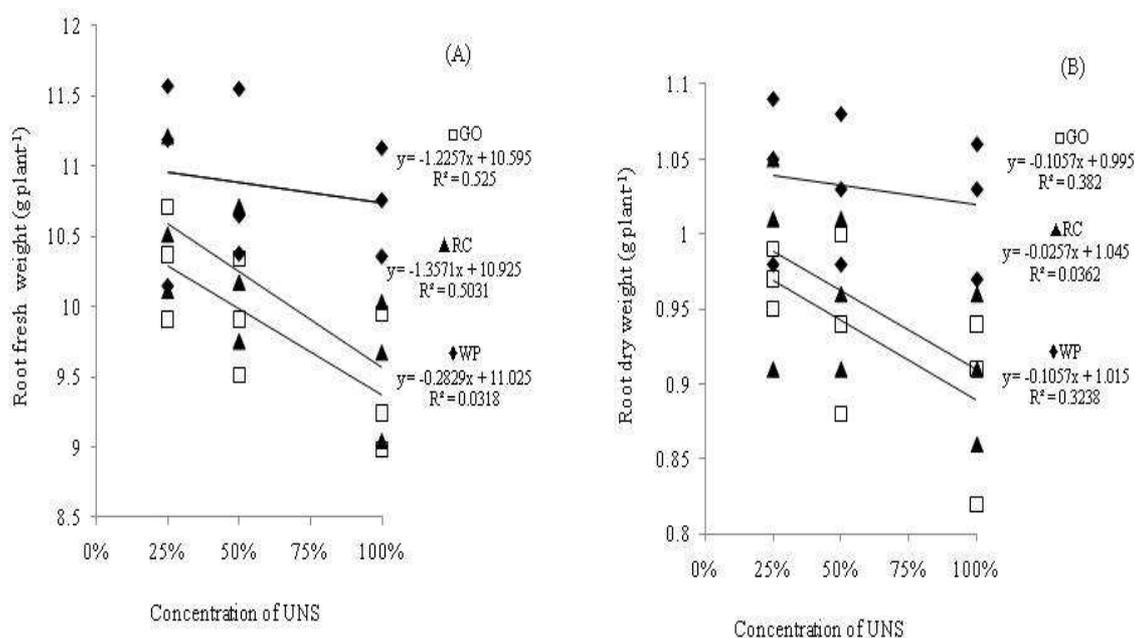
**Fig. 4:** Relationship between: (A) Root fresh weight; (B) Root dry weight of Green oak leaf lettuce grown in hydroponically and percentage of used nutrient solution (UNS) in nutrient solution derived from previously grown green oak leaf lettuce (GO), red coral lettuce (RC) and without plant (WP) in coconut-coir dust



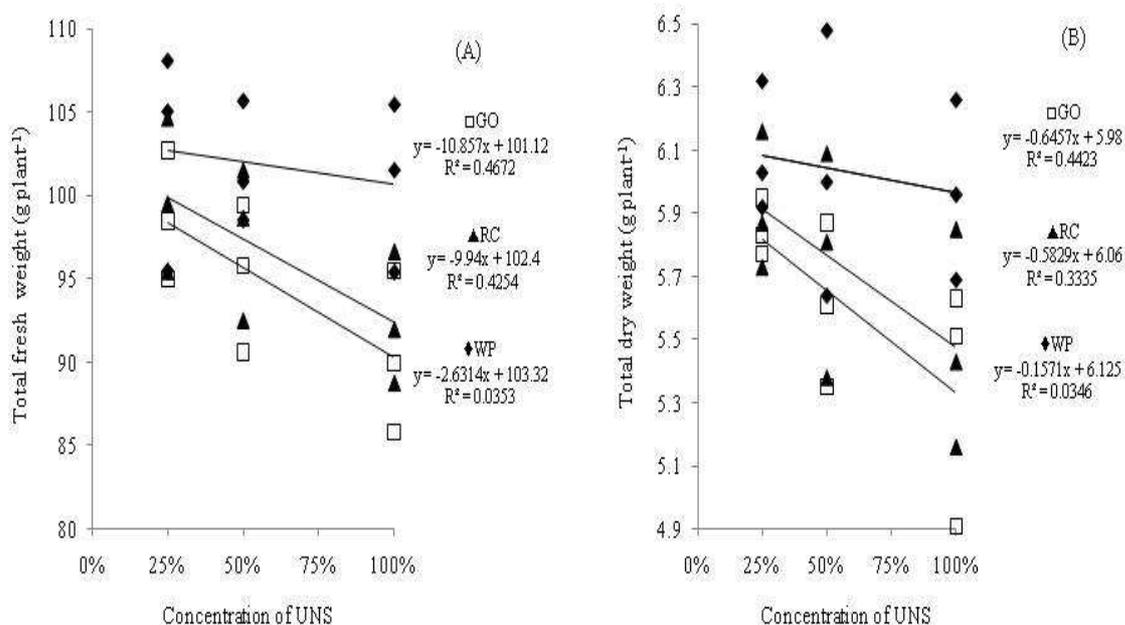
**Fig. 5:** Relationship between: (A) Total fresh weight; (B) Total dry weight of Green oak leaf lettuce grown in hydroponically and percentage of used nutrient solution (UNS) in nutrient solution derived from previously grown green oak leaf lettuce (GO), red coral lettuce (RC) and without plant (WP) in coconut-coir dust



**Fig. 6:** Relationship between: (A) Shoot fresh weight; (B) Shoot dry weight of Green oak leaf lettuce grown in hydroponically and percentage of used nutrient solution (UNS) in nutrient solution derived from previously grown green oak leaf lettuce (GO), red coral lettuce (RC) and without plant (WP) in hydroton



**Fig. 7:** Relationship between: (A) Root fresh weight; (B) Root dry weight of Green oak leaf lettuce grown in hydroponically and percentage of used nutrient solution (UNS) in nutrient solution derived from previously grown green oak leaf lettuce (GO), red coral lettuce (RC) and without plant (WP) in hydroton



**Fig. 8:** Relationship between: (A) Total fresh weight; (B) Total dry weight of Green oak leaf lettuce grown in hydroponically and percentage of used nutrient solution (UNS) in nutrient solution derived from previously grown green oak leaf lettuce (GO), red coral lettuce (RC) and without plant (WP) in hydroton

*Relationships among growth parameters affected by used nutrient solution:*

Linear regression was used to investigate the relationships among growth parameters affected by UNS in lettuce, under different concentration of UNS from coconut-coir dust and hydroton with green oak leaf lettuce, red coral lettuce and without-plant showed strong relationship (Fig. 3 to 8). The relationship indicates that shoot, root and total fresh and dry weight of Green oak leaf lettuce linearly decreased with proportional increases of used nutrient solution (UNS). Progressive decrease of UNS induces linear increases of shoot, root and total fresh and dry weight that means higher concentration of UNS may inhibit the fresh and dry weight of lettuce.

*Conclusions:*

Reuse of used nutrient solutions may increase the nutrient use efficiency, decrease environmental pollution and promote the economic advantage. From the results, percent growth reduction of Green oak leaf lettuce was lower in used nutrient solution (UNS) from hydroton and UNS from red coral lettuce exhibit its better performance. The results showed that, 25% used nutrient solution (UNS) mixing exhibited the better performance across the growing media compared with other treatments and its growth was comparable to from Enshi nutrient solution. Comparing between growth reduction from the best treatment (25% mixing), saving 25% of Enshi nutrient solution (ENS) which is more economical worth. The high pH and EC value of the ENS and UNS is needs to adjust daily basis for successful lettuce cultivation. The output of the findings from this study will be used for properly managing the used nutrient solution as to increase the water and mineral use efficiency in substrate culture in the near future.

**ACKNOWLEDGEMENTS**

The authors gratefully acknowledge the support from 'National Agricultural Technology Project: Phase-1' of Bangladesh Agricultural Research Council (BARC), Bangladesh funded by World Bank.

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