GROWTH AND QUALITY CHARACTERISTICS OF SPINACH (*Spinacia oleraceae* L.) IN VARIOUS SUBSTRATE CULTURES

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Abstract

The objective of this study was to evaluate the growth and quality characteristics of spinach on various substrates in a hydroponic growing system. The experiment was carried out with 4 treatments in 4 replicates, namely: (1) sponge, (2) coconut fibre, (3) mixture ratio of coconut fibre and husk ash (1:1), and (4) straw. The experimental results showed that the yield of spinach sown on both of sponge and coconut fibre were 24.6 times higher than those sown on straw substrate under the same hydroponic solution. The quality of spinach sown on the sponge substrate is also improved and get in line with the safe vegetable quality standards of the Ministry of Health.

Keywords: *Hydroponic substrates, spinach, spinach quality, yield.*

ĐẶC TÍNH SINH TRƯỞNG VÀ CHẤT LƯỢNG RAU CẢI BÓ XÔI (*Spinacia oleraceae* L.) TRÊN CÁC GIÁ THỂ KHÁC NHAU

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Tóm tắt

Mục tiêu nghiên cứu là đánh giá đặc tính sinh trưởng và chất lượng cải bó xôi được gieo trên các giá thể khác nhau trong hệ thống trồng rau thủy canh. Thí nghiệm được thực hiện với 4 nghiệm thức với 4 lần lặp lại bao gồm: (1) Giá thể bông mút, (2) Giá thể mụn xơ dừa, (3) hỗn hợp tro trấu và xơ dừa (tỉ lệ 1:1), và (4) Giá thể rơm. Kết quả thí nghiệm cho thấy năng suất năng suất cải bó xôi được gieo trên 2 giá thể bông mút và mụn xơ dừa đạt cao khác biệt gấp 24,6 lần so với cải bó xôi được gieo trên giá thể rơm trong cùng điều kiện dung dịch thủy canh. Chất lượng cải bó xôi được gieo trên giá thể bông mút cũng được cải thiện và phù hợp với tiêu chuẩn về chất lượng rau an toàn của Bộ Y tế.

Từ khóa: *Giá thể thủy canh, cải bó xôi, chất lượng cải bó xôi, năng suất.*

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1. Introduction

Spinach plays an important role in the diet and is consumed in great quantities all year around. Moreover, it is low in calories and fat but is rich in minerals, fiber, vitamins, and phytochemicals (Alvino & Barbieri 2016) and has high antioxidant activity (Ismail et al., 2010; Ou et al., 2002). Bioactive phytochemicals, ubiquitously distributed in nature, may constitute pigments that give a colorful appearance to foods, or secondary metabolism products that protect plant species from environmental attacks and increase their functional and nutritional values (Ismail et al., 2010). However, phytochemical accumulation and antioxidant activity are influenced by cultivation techniques (Ou et al., 2002), in which spinach production are changing.

In addition, soilless culture, including aeroponics, aquaponics, and hydroponics, is considered one of the most innovative agricultural strategies to produce more from less, in order to feed the estimated 11 billion people in the world by 2100 (Machado et al., 2018; Shete et al., 2017). Spinach production in soilless cultivation systems, mainly in substrate, using various substrate in plant hydroponic is increasing worldwide (Machado et al., 2018).

Hydroponic culture is a cheap and easy option for organic vegetable production. It is a technique that involves growing plants in water using mineral nutrients without soil (Diver & Rinehart, 2006; Sapkota et al., 2019; Wahome et al., 2011). Easy control of nutrient composition, lack of soil contamination, faster plant growth, shorter crop cycles, high product quality, and good consumer acceptance have made hydroponics an important alternative plant production technique (Benke & Tomkins 2017; Rothwell et al., 2016).

Even though hydroponic culture can produce optimal plant growth (better yield and quality), its efficiency depends on many factors such as nutrient availability, crop genotype, growing method, and pest management (Sapkota et al., 2019). Therefore, this paper is aimed to assess spinach growth and yield on diverse substrate cultures in hydroponic system.

2. Materials and methods

2.1. Materials

- Styrofoam planting boxes (39 cm long, 25 cm wide and 30 cm high) were filled with 10 L of substrates. Hydroponic Styrofoam containers (49 cm long, 29 cm wide and 33 cm high) were filled with 20 L of water and mineral fertilizers and plastic cups (height 9 cm and width 4.5 cm).

- Various substrates were used: Sponge, coconut fibre, mixture of Coconut fibre and husk ash (at ratio of 1:1) and straw.

- Spinach seeds were bought from Phu Nong Co., Ltd.

- The used hydroponic nutrient solution was the extracted solution from seafood sludge compost (SSC) researched result by Nguyễn (2020). The contents of major nutrients in extracted solution of seafood sludge compost consisted of 400 ppm K, 80 ppm Ca, 80 ppm Mg, 300 ppm NO_3^-, 400 ppm $H_2PO_4^-$. Electrical conductivity (EC) of solution was 498 µS/cm and pH 6.5.

2.2. Methodology

2.2.1. Experimental design

The experiment was conducted in a completely randomized design with 4 treatments in four replications. Each replication was carried out in 12 plastic cups with three plants for each cup. All treatments were described in Table 1 below.

Table 1. The experimental treatment

No. of treatment	Substrate
T1	Sponge (Control)
T2	Coconut fibre
T3	Coconut fibre and husk ash (1:1)
T4	Straw

2.2.2. Growth conditions and substrates

Substrates were the following sponge, coconut fibre, coconut fibre and husk ash (1:1 v/v), and straw. The 4 cm thick sponge was shortened to 4x5 cm diameter. Straw was also cut about 2 cm. Coconut fibre was soaked with lime at 5% concentration during 24 hours. Then

it was washed with fresh water and then lightly dried under the Sun. Husk ash was washed with fresh water twice.

Spinach (*Spinacia oleraceae* L.) seedlings (three seedlings per plastic cup) were transplanted at 7 days after emergence in Styrofoam box. After transplanting, the plants were supplied with the nutrient solution weekly. During the culture of spinach plants, insect pests were also controlled.

When spinach grew from 7 to 15-day-old seedling plant, the amount of hydroponic nutrient solution was 10 ml extracted solution from SSC through a 1-L volume of fresh tap water. From the post-15-day planting on, the amount of nutrient solution was 20 ml from SSC through a 1-L volume of fresh tap water.

The pH of the nutrient solution was maintained between 6.5 and 7.5 by adding NaOH or HCl as per need. The pH and EC of the solution was checked three times a week. Furthermore, 2 L of water and nutrient solutions were added to replenish each hydroponic solution 7 days after treatment initiation.

Data collection

Spinach plants were harvested 30 days after treatment initiation. The response variables measured were plant height, fresh weight, root weight, number of leaves and quality characteristics (such as total sugar content (% Brix), nitrate, heavy contaminants (i.e. Pb and Cd), and human pathogenic microorganisms (i.e. *E.Coli* and *Salmonella*). The number of leaves and plant height were collected at sowing days of 5, 10, 15, 20, 25 and 30. The others were collected at the end of sowing (at the harvest time).

2.2.3. Statistical analysis

All analyses were carried with four replicates per sample, and mean results per sample were used for statistical data treatment. ANOVA statistical calculations were carried out using software SPSS version 20.0. Significant differences between all treatments were analyzed by the Duncan when $p<0.05$. All parameters such as plant height, fresh weight, root weight, number of leaves and quality characteristics were analyzed for significantly statistical differences.

3. Result and Discussion

3.1. Effect of different substrates on spinach growth and yield

3.1.1. Overview

Effect of nutrient solution from seafood sludge organic compost on spinach growth and yield were described in Figures 1 and 2. Generally, criteria of plant height, number of leaves and root length of spinach were not significantly different between treatments in the early stage (from 0 to 10-day-old seedling plant).

However, from the 10-day sowing to harvest stage, spinach grown in sponge, coconut fibre and mixture of coconut fibre with husk ash (1:1) gave production criteria higher values than treatment of straw culture. When spinach used nutrient solution from extracted SSC got thickest leaf and darkest color.

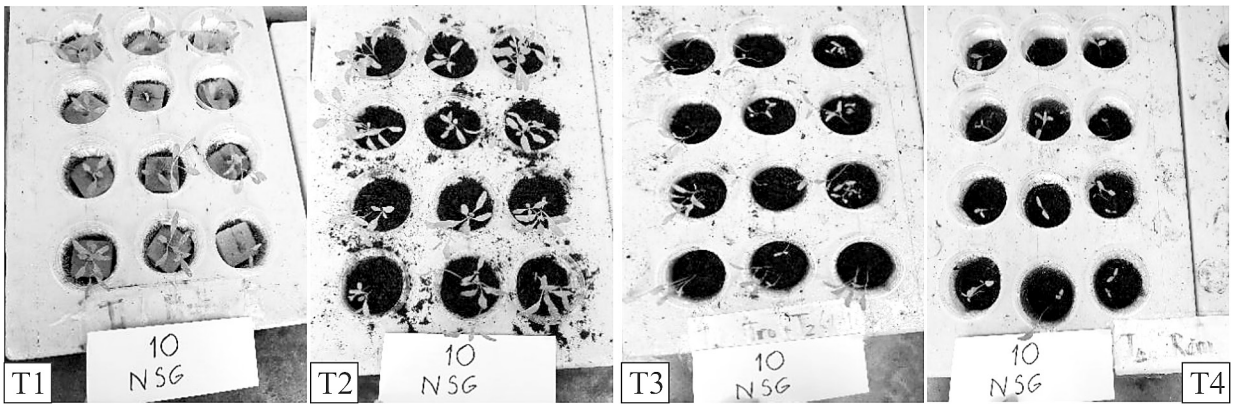

Figure 1. Ability of Spinach growth in initial period (after 10-day sowing)

Figure 2. Ability of spinach growth in harvest period (after 30-day sowing)

3.1.2. Spinach growth characteristics

a. Number of leaves

The number of leaves of spinach was recorded at 5, 10, 15, 20, 25 and 30 days after sowing. Significant variation in number of leaves was observed among the treatments. Spinach cultivar grown in the T1, T2 and T3 substrates generally had a significantly greater number of leaves compared to the other one (T4 media culture) (Figure 3). The highest number of leaves was obtained with sponge, followed by coconut fibre and mixture between coconut fibre and husk ash and the lowest number of leaves was found with straw culture during plant growth period. Differences in the number of leaves per plant could be associated with either substrate composition and/or plant characteristics.

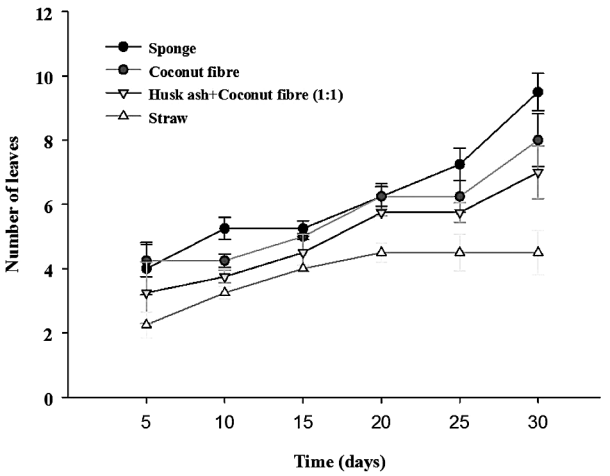

Figure 3. Effects of different hydroponic substrates on the number of spinach leaves

Note: The vertical bars represent the standard error (n=4).

The percent increase in number of leaves recorded with sponge substrate compared to straw media culture was 9.5 and 4.5 at 30 days after sowing, respectively. However, the result showed that there were no statistically significant differences in leaf number between the T2 and T3 substrates.

b. Plant height

The growth of spinach in terms of shoot height in different treatments is shown in Fig. 4. Plant height among the treatments varied from 8.57 to 19.13 cm and 8.99 to 21.53 cm at 25 and 30 days after sowing, respectively. Throughout the supply in hydroponics culture, the highest plant height (21.53 cm) was obtained when spinach was grown in sponge substrate while the lowest plant height was found with T4 treatment during the growing periods. Similar results were found with application of coconut fibre substrate up to 19.75 leaves at 30-day-old seedling plant, higher than T4 substrate treatment. However, there were no significant differences in plant height among the treatments T1 and T2 throughout the period. Plant height was increased by 58% and 54% with T1 and T2 substrates compared to T4 at 30-day-old seedling plant, respectively. This investigation could be attributed a significant ($P < 0.05$) difference in plant height probably due to better physical environment in terms of plants grown under different substrates. This is in agreement with Shinohara et al. (2011) who asserted that using organic residues to make nutrient media culture could provide enough macro-nutrients and micro-nutrients. This extra

finding also indicated that straw substrate can limit spinach growth capacity because of organic non-decomposition. As a result, it is impossible that spinach root can uptake nutrient from hydroponic solution.

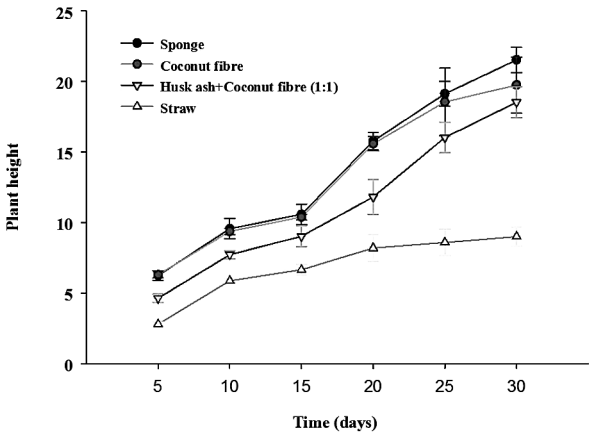

Figure 4. Effects of different hydroponic substrates on spinach height

Note: The vertical bars represent the standard error (n=4).

c. Root length

There were significant differences in root length among spinach grown in the different nutrient substrates. The T1 substrate generally produced the longest roots (31.03 cm), whereas those grown in T4 had the shortest roots (Table 2). The cause of longer roots could be nutrient uptake ability of root likely due to lower mineral availability in the diverse planting cultures. Fraile-Robayo et al. (2017) found that the growth of vegetable in a hydroponic system and root size depended on nutrient solution composition, substrates, water availability and temperature.

d. Fresh weight and yield

Spinach vegetable had the greatest fresh weight in the T1 and T2 amended substrate, followed by T3 and T4 substrates (Table 2). There was a statistically significant difference among treatments for fresh weight. Interestingly, amended layers of sponge and coconut fibre resulted in full-grown plants of marketable size with 114.22 g/plant and 113.66 g/plant, respectively. The T4 substrate produced the lowest fresh weight compared to the other treatments (Table 2). Thus, yield variation in spinach could be also fluctuated toward various types of substrates in the hydroponic cultivation. The highest remarkable yield of spinach came from T1 and T2 substrates, making 2515.06 g/box and 2502.85 g/box, respectively. These were statistically significant differences to the other ones. The lowest yield of spinach was seen in T4 substrate, reaching at 102.72 g/box.

Table 2. Effects of various hydroponic substrates on spinach growth and yield

Treatment	Fresh weight (g/plant)	Yield (g/box)	Root weight (g/root)
T1 - Sponge	114.22[a]	2515.07[a]	31.03[a]
T2 - Coconut fibre –	113.66[a]	2502.85[a]	30.73[a]
T3 - Husk ash + Coconut fibre (1:1)	110.61[b]	2435.69[b]	22.13[b]
T4 - Straw	4.67[c]	102.72[c]	17.95[c]
CV (%)	1.66	1.66	12.84

Note: Means followed by same letter along columns for each hydroponics substrate were not significantly different at 5% level.

A previous study by Sapkota et al. (2019) suggested that diverse nutrient content of substrate can be the primary factors that influence plant growth and biomass production in hydroponic culture.

Overall, the results indicated that T1 and T2 were suitable substrates for spinach growth. Both substrates gave fresh weight and yield as well as number of leaves and root length which were higher than T3 and T4 amended treatments.

3.2. Effects of various substrate cultures on quality characteristics of spinach

3.2.1. Total sugar content (%Brix)

The research results reported that the spinach quality was at the highest total sugar amount of 3.75% in spinach sown on sponge substrate culture, whereas it decreased with the other ones (Table 3). The increase may be attributed to the hydrolysis of starch/sucrose into sugar and the increase in reducing sugar content during storage (Bhardwaj & Pandey, 2011).

3.2.2. Vitamin C

Ascorbic acid (vitamin C) is the micro nutrient most readily associated with vegetables (and fruit). Vitamin C content varies considerably between different vegetables even within a particular vegetable type. Thus, the level of vitamin C is not an indicator of quality per SE. However, since the vitamin is vulnerable to chemical and enzyme oxidation, and is highly water soluble, it is a sensitive and appropriate marker for monitoring quality change during transportation, processing, and storage (Favell, 1998; Oboh & Akindahunsi, 2004). Vitamin C content on all the substrates ranged from 77.6 to 79.2 mg/kg but was not statistically different among them (Table 3).

Table 3. Effects of various hydroponic substrates on spinach qualities

Treatment	Brix (%)	Vitamin C (mg/kg)	Nitrate (mg/kg)	Pb (µg/kg)	Cd (µg/kg)	E.Coli (CFU/g)	Salmonella (CFU/g)
T1	3.75^{a}	78^{ns}	4050^{a}	85^{b}	ND	10^{ns}	ND
T2	3.03^{b}	77.5^{ns}	3165^{b}	91.2^{ab}	ND	10^{ns}	ND
T3	2.85^{b}	79.2^{ns}	3752^{a}	95.2^{a}	ND	9^{ns}	ND
T4	2.70^{b}	-	-	-	-	-	-
CV (%)	**7.54**	**1.66**	**1.66**	**12.84**		**7.54**	

Note: Means followed by same letter along columns for each hydroponics substrate were not significantly different at 5% level. T1: Sponge (control), T2: Coconut fibre, T3: Husk ash + Coconut fibre (1:1), T4: Straw, ND: not detected, "-": not data.

3.2.3. Nitrate

Spinach grown in both sponge and mixture of husk ash and Coconut fibre (1:1) substrates had the highest nitrate content (4050 mg/kg). Nitrate content of spinach was lowest at Coconut fibre substrate (3752 mg/kg). Spinach sown on straw substrate data was not recorded due to its lowest yield (Table 2). Total fresh weight of spinach sown on this substrate was not enough quantity to identify the quality indicators. Therefore, the researched results of this case were not presented and discussed. In Vietnam, there are standard limitations of nitrate content for some types of vegetables and fruits, except spinach. In addition, a survey conducted by the UK Ministry of Agriculture, Fisheries and Food (MAFF) revealed that spinach had relatively high nitrate concentrations (greater than 1000 mg/kg). The results of the study revealed that leafy vegetables to contain higher levels of nitrates and among them, the highest value was recorded in spinach, which was 5830 ppm (Ranasinghe and Marapana 2018). Therefore, this survey is consistent with the researched results of this experiment.

3.2.4. Heavy contaminants and human pathogenic microorganisms

The results of the study shown in Table 3 indicated that population of human pathogenic bacteria such as E.Coli and Salmonella in spinach grown on all three substrates of sponge, coconut fiber and mixture of coconut fiber and husk ash (1:1) reached off allowed standard according to QCVN 8-3: 2012 /BYT.

This result was similarly observed for heavy metal contaminants (Pb and Cd) in spinach. Heavy metal content in all treatments ranged from 85 - 95.2 µg/kg for Pb content. The Pb content level of spinach was highest to grow on mixture of coconut fibre and husk ash and the lowest being the treatment grown on sponge. However, these heavy contaminants were below limited regulation in QCVN 8-3: 2012/ BYT (i.e.

Pb < 0.3 mg/kg). The cadmium (Cd) content in spinach was not detected, conforming to Vietnamese limited standards (i.e.QCVN 8-3: 2012/BYT and QCVN 8-2: 2011/BYT).

In general, the nutrient quality of spinach grown on the four kinds of substrates fluctuated sharply and differed statistically, except human pathogen microorgism and heavy contaminants.

4. Conclusions

Spinach sown on both of sponge and coconut fibre substrates achieved the highest indicators in yield. The leaves' number, plant height, root length and yield of spinach grown on sponge and coconut fibre were 9.5 and 8 leaves, 21.53 and 19.75 cm, 31.03 and 30.73 cm, 2515.07 and 2502.85 g/box, respectively. These yields were 25 times higher than that of straw substrate.

However, the spinach quality characteristics were not much different among the substrates. All the quality indicators were conformed to Vietnamese limited standards.

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