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Nitrate control and quality in hydroponic lettuce by using cow dung extract and nutrient solution

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Summary

Hydroponically grown lettuce poses a high nitrate risk, raising concern about its quality. Therefore, this work was employed to determine the accumulation of nitrate in lettuce leaf and the phytochemical properties of hydroponic lettuce. Treatments considered as four different levels of aerated cow dung extracts (CD), *viz.*, CD_1 = 50 g·L^{-1}, CD_2 = 100 g·L^{-1}, CD_3 = 150 g·L^{-1} and CD_4 = 200 g·L^{-1} and four strengths of standard nutrient solution (S), *viz,* S_1 = 30% of standard nutrient solution, S_2 = 40% of standard nutrient solution, S_3 = 50% of standard nutrient solution and S_4 = 60% of standard nutrient solution. The experiment was carried out in a deep flow technique in semigreenhouse. In the case of cow dung extract, the highest total fresh weight (112.05 g/plant) was recorded from CD_3 while the lowest in CD_1 and for nutrient solution, the highest fresh weight (116.0 g/plant) was recorded from S_4 while the lowest in S_1. In the event of, nitrate and ascorbic acid content were statistically higher in CD_4 followed by CD_3 and the lowest in CD_1. In case of nutrient solution, the nitrate content was highest in S_4 and the lowest in S_1. The highest fresh weight and almost all the parameters were found to be the best in CD_3S_4 and the lowest in CD_1S_1. Therefore, the analysis showed that CD_3S_4 would be the most preferable treatment combination for producing quality lettuce with the lower content of nitrate.

Keywords: Nutrient solution, cow dung extract, nitrate control and hydroponic lettuce

Introduction

Hydroponics, as a high-potential cultivation technique is getting global attention and experiencing an increase in production of vegetables like lettuce (*Lactuca sativa* L.). Although growing lettuce without soil can provide higher yield, and nutritionally superior produce (MAJID et al., 2021) there are still nitrate risk issues because lettuce has a predisposition to nitrate accumulation (DAIANE et al., 2021). High levels of nitrate in lettuce are undesirable because excessive nitrate in plant is harmful to plant growth as well to human health (KHAN et al., 2018). The nitrate issue has now become to be a serious concern for consumers because lettuce is frequently eaten fresh as a salad vegetable due to a wonderful source of vitamins and minerals (STAGNARI et al., 2015). The European Union and the WHO have therefore recommended upper limits for NO_3^- concentration in greenhouse-produced lettuce leaves for winter and summer crops were set at 5000 and 4000 mg kg^{-1} fresh weight, respectively (OFFICIAL JOURNAL OF THE EUROPEAN UNION, 2011).

Abundant nitrate availability leads to excessive absorption by the roots in larger quantities resulting in nitrate accumulation in the vacuoles of the cells (COLLA et al., 2018). Nitrate accumulation by leafy crops is strongly correlated with the compositional amount of nitrogen fertilizer (CHEN et al., 2014). Though ammonium (NH_4^+) and nitrate (NO_3^-) are two main nitrogen (N) forms that can be absorbed and utilized by plants (XU et al., 2012) but two forms are not independent of each other in terms of their uptake. Ammonium uptake can suppress nitrate uptake which has been demonstrated clearly in many leafy plants (FALLOVO et al., 2009). Therefore, manipulation of nitrogen fertilization in terms of application rate and source type appears as the most applicable means to prevent nitrate accumulation in plants (WANG et al., 2008). The supply of the N in the nutrient solution in the ammonium form, allowing a reduction in the accumulation of NO_3 - in the vacuoles (ANDRIOLO et al., 2006; ROCHA et al., 2020). Partial replacement of the nitrate fertilizer with ammonium is an advantageous practice to avoid nitrate accumulation without negative consequences on (BURNS et al., 2012; TSOUVALTZIS et al., 2014) and some plants prefer NH_4^+ (BRITTO and KRONZUCKER, 2013) such as lettuce, known as ammonium-philic plant (IKEDA and OSAWA, 1981). However, NH_4-N cannot be the major source of N as the current recommendation for soilless culture is that NH_4-N should not exceed 25% of the total-N supply (SONNEVELD, 2002). Recent research has proven that the use of organic fertilizers, such as cattle manure in lettuce crop production can be considered a valid and useful alternative source of organic nitrogen (MONTEMURRO, 2010) whereas the roots of a few plants absorb organic N directly (NÄSHOLM et al., 2009). Therefore, the use of liquid organic fertilizer such as cow dung extract as a partial replacement to mineral nutrients is an attractive solution for controlling NO_3^- accumulation in hydroponic lettuce. Moreover, consumers are demanding higher quality and safer food and highly interested in organic products (OUDA et al., 2008). Hence much attention has been paid in recent years to manage different organic waste resources to improve organic fertilizers through biological processes at low-input as well as an eco-friendly basis (SUTHAR, 2007). Organic fertilizer has been microbially preprocessed before incorporation into hydroponic solutions (ATKIN and NICHOLS, 2004). The pre-processing can generate 25 to 50% ammonium as an intermediate product through the ammonification of organic fertilizer which can be used efficiently in hydroponic (SHINOHARA et al., 2011) along with nutrient solution. However, a reduction of nitrate content can add value as it is an essential nutritional quality factor of vegetables (KOSSON et al., 2017) and this quality of lettuce can be influenced both by the mineral and organic fertilizer type (VILLAGRA et al., 2012). In this context, the present research was carried out to optimize NO_3^- concentration in lettuce grown in nutrient solution with cow dung extract. Therefore, the aims of the study were to find out the appropriate dose of cow dung slurry and its effects on NO_3^- concentration in lettuce leaf and the yield and quality of lettuce in a hydroponic system.

Material and methods

Experimental site and structure

Two years of repeated experiments were conducted, from September 2019 to March 2020 and from September 2020 to March 2021. It was conducted in the semi-greenhouse at the Horticulture Farm, Sher-e-Bangla Agricultural University, Dhaka, Bangladesh. The experiments were conducted in a structure using polyvinyl chloride (PVC)

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pipes. The structure consisted of four 5-foot lengths of 5-inch PVC pipe and a stand trellis made up of strong and durable steel. The stand, which measures 3 feet by 3 feet by 2.5 feet and houses four growing tubes, has been designated as an experimental unit (Plate 1). Holes were made on the upper part of the pipe, and the distance between two holes was 19.06 cm. Pipes had been placed horizontally on this stand as the holding plants became more exposed to sunlight.

Experimental design and treatment

The two factors experiment were conducted in a completely randomized design (CRD) with four replications. Factor A considered as four different types of cow dung extract denoted as CD, *viz.*, CD_1 = Cow dung extract 50 $g \cdot L^{-1}$, CD_2= Cow dung extract 100 $g \cdot L^{-1}$, CD_3 Cow dung extract 150 $g \cdot L^{-1}$, and CD_4 = Cow dung extract 200 $g \cdot L^{-1}$ and factor B considered as four different strengths of standard nutrient solution (Rahman and Inden (2012) used as standard nutrient solution) denoted as S, *viz.*, S_1 = 30% strength of standard solution, S_2 = 40% strength of standard solution, S_3 = 50% strength of standard solution, and S_4 = 60% strength of standard solution. Loose leaf type lettuce (*Lactuca sativa* cv. 'Green Wave') was used as a planting material and eight plants considered as an experimental unit (Tab. 1).

Tab. 1: Treatment concentrations of lettuce with cow dung or nutrient solution

CD_1	Cow dung extract 50 $g*L^{-1}$
CD_2	Cow dung extract 100 $g*L^{-1}$
CD_3	Cow dung extract 150 $g*L^{-1}$
CD_4	Cow dung extract 200 $g*L^{-1}$
S_1	30% strength of standard solution
S_2	40% strength of standard solution
S_3	50% strength of standard solution
S_4	60% strength of standard solution

Nutrient solution treatments:

Nutrient solution is the most important component of the hydroponic system and in this present study, the treatments nutrient solution was prepared by mixing modified hydroponic standard solution and cow dung extract. The nutrient solution was prepared with distilled water and chemical grade reagents. The ratio of Rahman and Inden (2012) solution were NO_3-N, P, K, Ca, Mg, and S of 17.05, 7.86, 8.94, 9.95, 6.0 and 6.0 meq. L-1, respectively. The rates of micronutrients were Fe, B, Zn, Cu, Mo and Mn of 3.0, 0.5, 0.1, 0.03, 0.025 and 1.0 mgL^{-1}, respectively for both the nutrient solutions. The cow dung extract was formulated by merging of following two different methods which are (Charoenpakdee, 2014; Peiris et al., 2015) where cow dung was used as a raw material organic source of nutrient and Mazim organic fertilizer (Mazim Agro Industries Ltd.) as a source of microbial inoculum.

pH and Electrical Conductivity of solution:

The pH and EC values for all nutrient media were determined prior to use. The EC of each nutrient solution was about 2.0 dS /m, and the pH was adjusted at 5.5 to 6.5 using citric acid for the organic nutrient solutions, but for the inorganic solution the pH was adjusted by using nitric and phosphoric acids (3: 1 v/v).

Harvesting and data collection

At random three loose leaf lettuce plants from each treatment were harvested after 42 days of sowing. The chlorophyll concentration was estimated at harvest in the second leaf using Minolta chlorophyll Meter SPAD -501 plus, since it was portable and chlorophyll concentration can be estimated nondestructively. After harvesting substrate of cultivation treatment were gently washed off. The fresh weight of the whole plant, leaves, and roots was recorded for each plant with an analytical scale immediately after removal the free surface moisture with soft paper towel. Using the 2,6-dichlorophenol indophenol (DCPIP) visual titration method, the ascorbic acid concentration (ascorbic acid) was determined (Rangana, 2004). Estimation of beta-carotene using the calibrated reflection spectroscopy method (Masoumi et al., 2018). Estimation of NO_3 content and nitrate percentage on the edible part of lettuce using the ion chromatographic method (Thabano et al., 2004). The estimation β-Carotene, ascorbic acid and NO_3 content of nitrates percentage on edible part of lettuce was made in the Bangladesh Council of Science and Industrial Research (BCSIR).

Statistical analysis:

The data obtained for different parameters were statistically analyzed with SPSS version 26.0 and means separation were done by Tukey's test at $P \leq 0.05$.

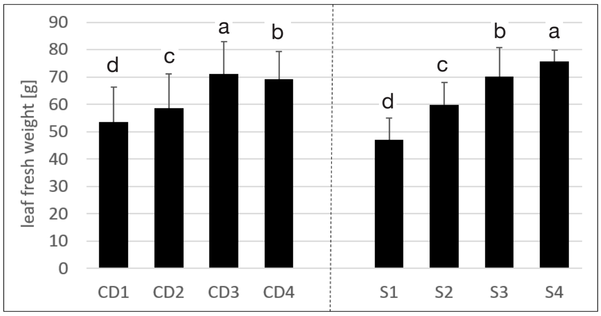
Results and discussion

Fresh weight of lettuce

Marketable lettuce quality is determined primarily by the size of the plant and its fresh weight. insignificant difference in fresh weight at transplanting time but differed at harvesting among the treatments (Fig. 2 and Fig. 3).

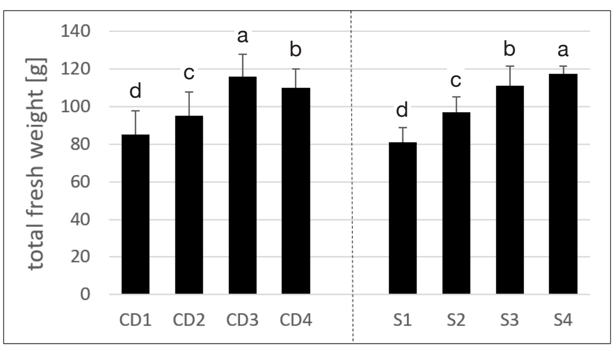
The total fresh weight increased as the number of days until maturity. At harvest time, for cow dung extract, total fresh weight was found to be higher in CD_3 (116.0 g/plant) than in CD_1 (85.19 g/plant), and for nutrient solution, it was found to be higher in S_4 (112.05 g/plant) than in S_1 (80.91 g/plant). In response to cow dung extract, the highest fresh weight of leaf (71.02 g/plant) was found in CD_3, and the lowest was found in CD_1 (53.63 g/plant), and for the nutrient solution, the highest and lowest weights were found in S_4 (75.73 g/plant) and S_1 (46.99 g/plant), respectively. It was revealed that balanced nutrition and the optimum level of nitrogen ensured maximum vegetative growth, resulting in the highest fresh weight per plant. The results obtained earlier by (Tittonell et al., 2003), were similar to the present study. In all cases, the highest fresh weight was found in CD_3S_3, which was statistically similar to CD_4S_4, and the lowest was found in the CD_1S_1 combination. Studies have revealed that co-provision of NH_4^+ and NO_3^- nutrition significantly stimulated plant growth in comparison with the addition of NH_4^+ or NO_3^- alone (Song et al., 2017; Zhu et al., 2018).

Fig. 1: Growing lettuce plants on a hydroponic structure



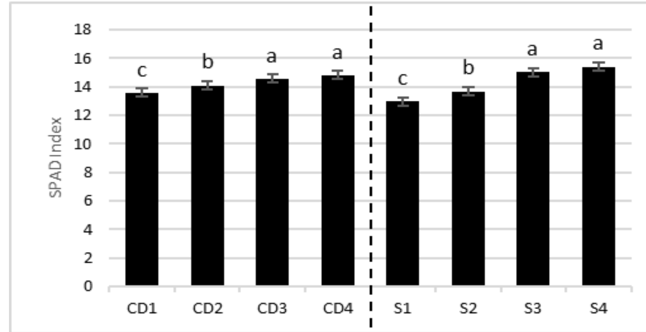
	df	*SS*	*MS*	*F*	*P*-Value
Cow dung					
Extract (CD)	3	5759.9	1919.9	1480.8	0.002
Nutrient Solution (S)	3	2528.1	842.71	649.96	<0.001
CD × S	9	317.0	35.22	27.16	<0.001
Error	32	41.49	1.29		

Fig. 2: Effects of cow dung extract and nutrient solution on leaf fresh weight of lettuce.
Bars represent means with standard deviation (n=3). Different letters atop the bars indicate significant differences according to Tukey's test at $P \leq 0.05$. Outcome of two-way ANOVA is represented in the table.



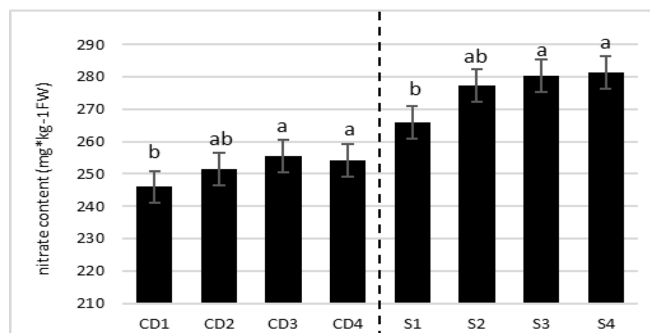
	df	*SS*	*MS*	*F*	*P*-Value
Cow dung					
Extract (CD)	3	9540.8	3180.2	1949.4	<0.001
Nutrient Solution (S)	3	7067.7	2355.9	1444.1	<0.001
CD × S	9	547.1	60.79	37.26	<0.001
Error	32	52.2	1.63		

Fig. 3: Effects of cow dung extract and nutrient solution on total fresh weight per leaf of lettuce.
Bars represent means with standard deviation (n=3). Different letters atop the bars indicate significant differences according to Tukey's test at $P \leq 0.05$. Outcome of two-way ANOVA is represented in the table.



	df	*SS*	*MS*	*F*	*P*-Value
Cow dung					
Extract (CD)	3	10.632	3.544	2.728	0.055
Nutrient Solution (S)	3	47.865	15.955	35.229	<0.001
CD × S	9	60.517	4.034	17.746	<0.001
Error	32	7.275	0.227		

Fig. 4: Effects of cow dung extract and nutrient solution on total SPAD values of lettuce.
Bars represent means with standard deviation (n=3). Different letters atop the bars indicate significant differences according to Tukey's test at $P \leq 0.05$. Outcome of two-way ANOVA is represented in the table.



	df	*SS*	*MS*	*F*	*P*-Value
Cow dung					
Extract (CD)	3	822.73	274.24	17.20	0.001
Nutrient Solution (S)	3	619.58	206.52	12.96	<0.001
CD × S	9	104.58	11.62	0.729	<0.001
Error	32	510.00	15.93		

Fig. 5: Effects of cow dung extract and nutrient solution on nitrate content in lettuce.
Bars represent means with standard deviation (n=3). Different letters atop the bars indicate significant differences according to Tukey's test at $P \leq 0.05$. Outcome of two-way ANOVA is represented in the table.

SPAD index and nitrate content on lettuce

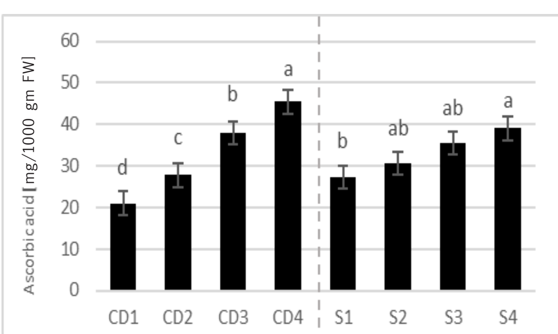
Nitrate accumulation by plants is observed when plant nitrogen uptake exceeds the assimilation capacity. The SPAD index is the N nutrition status indicator of crops. It is widely used to monitor leaf N status of many crops, including lettuce. With the application of different levels of cow dung extract and nutrient solution, significant variations were found in SPAD index value and nitrate content of lettuce (Fig. 4 and 5). Thus, the highest SPAD index value was observed in CD_4 which was statistically same with CD_3. On the other hand, the lowest SPAD index value observed in CD_1. As for nitrate content, in case of cow dung extract maximum nitrate was determined in CD_4 which was statistically similar with CD_3 and the lowest was in CD_1. These may be due to the amount and composition of the nitrogen. Analysis of representative cow dung slurry samples made at the Bangladesh Agricultural Research Institute (BARI) and Dhaka University (DU) has shown that the slurry contains a considerable amount of both macro and micronutrients besides appreciable quantities of organic matter (ISLAM, 2006). The release of nitrogen in cow dung is slower than that in nutrient solution since organic fertilization typically does not provide nitrogen in a readily accessible form. HERENCIA et al. (2011) also reported similar results earlier. In case of nutrient solution, the highest SPAD value was observed in S_4 which was statistically same with S_3 where the lowest SPAD value was in S_1. For nitrate content was lowest nitrate was determined in S_1 and the highest nitrate content on lettuce was determined in S_4 and it was statistically similar with S_3. Like the studies by (Chen et al., 2004; Petropoulos et al., 2008) this experimental finding revealed that, accumulation of nitrate closely related to the amount of fertilizer added. The interaction between different levels of cow dung extract and nutrient significantly affected SPAD index and nitrate content of lettuce leaf. The lowest SPAD value was observed in CD_1S_1 and the highest value was observed in CD_4S_4. The higher nitrogen doses significantly increased SPAD index values. The maximum nitrate content was determined in CD_4S_4 (265.24 mg kg^{-1} FW) which is statistically and similar with the treatments CD_4S_3 (263.48 mg kg^{-1} FW) and CD_3S_4 (262.24 mg kg^{-1} FW). The lowest nitrate content was determined in CD_1S_1 (245.95 mg kg^{-1} FW). However, the relatively low nitrate contents reported here and even the highest leaf nitrate content observed here was far below the limit recommended by the European Union (3500 to 4500 mg kg^{-1} of fresh matter) that were likely to be related to the environmental conditions of this work, especially high luminosity and temperature. Studies have revealed that co-provision of NH_4^+ and NO_3^- nutrition significantly stimulated plant growth in comparison with the addition of NH_4^+ or NO_3^- alone and reduced the content of nitrates (Song et al., 2017; Zhu et al., 2018).

Ascorbic acid content

Ascorbic acid content of lettuce was significantly affected by addition of different levels of cow dung extract (Fig. 6). In case of cow dung extract the highest ascorbic acid content of lettuce was found in CD_4 (45.41 mg/1000 gm FW) and the lowest ascorbic acid content of lettuce was found in CD_1 (21.00 mg/1000 gm FW). In this present experiment, it was observed that, ascorbic acid content increased with increasing levels of cow dung extract. Ascorbic acid content of lettuce was significantly affected by addition of modified strength of nutrient solution (Fig. 6). For nutrient solution, the highest ascorbic acid content of lettuce was found in S_4 (35.41 mg/1000 gm FW) and the lowest ascorbic acid content of lettuce was found in S_1 (27.25 mg/1000 gm FW). Shinohara et al. (1981) reported that ascorbic acid content of lettuce was increased when grown in ¼ strength nutrient solutions compared to the ½ strength nutrient solutions. In this experiment, the content of ascorbic acid increased with an increased concentration of nutrient solution treatment of S_4 (60% of the standard solution) that was compatible with these results. There was a significant interaction between cow dung extract and nutrient solution in case of ascorbic acid concentration on lettuce. The lowest ascorbic acid content of lettuce was found in C_1S_1 (18 mg/1000 gm FW). On the other hand, the highest ascorbic acid content of lettuce was found in C_4S_4 (50 mg/1000 gm FW) and the relatively similar ascorbic acid concentration in C_4S_3 (47 mg/1000 gm FW) and C_3S_4 (47 mg/1000 gm FW) which are statistically same. The results of present study revealed ascorbic acid content increased markedly with the increasing levels of nutrient solution with cow dung extract because the mixture of NH_4^+ and NO_3^- increased the content of soluble sugars, soluble proteins, and vitamin C in plants (Tabatabaei et al., 2008).

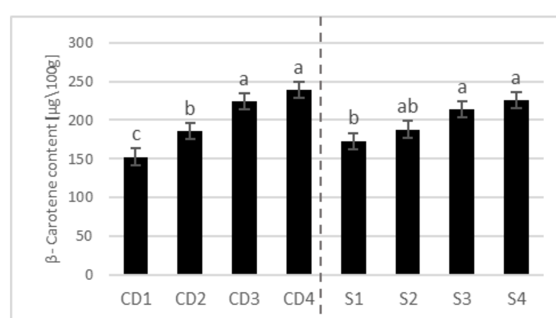
β-Carotene content

β-Carotene content in lettuce plant increased with the increasing levels of cow dung extract (Fig. 7). β-Carotene content was higher in the plants grown in CD_4 (239.00 µg/100 g) on the other hand the lowest β-carotene content of lettuce was estimate in CD_1 (152.53 µg/100 g). Ismail and Cheah (2003) showed that many organically grown vegetables were higher in vitamins than that conventionally grown and the findings of β-carotene content was 2006 µg/100 g. In the present study also reported similar findings. β-Carotene content of lettuce was significantly affected by addition of modified strength of nutrient solution (Fig. 7). For nutrient solution, the highest β-carotene content of lettuce was estimate in S_4 (226.17 µg/100 g) and the lowest in S_1 (173.00 µg/100 g). β-Carotene content of lettuce of lettuce had been substantially affected by the combination of cow dung extract and nutrient solution treatment. The lowest β-carotene content observed in CD_1S_1 and the highest value was observed in C_4S_4 (258.33 µg/100 g). It was showed that the maximum β-carotene content determined in C_4S_4 relatively similar with the treatments



	df	*SS*	*MS*	*F*	*P*-Value
Cow dung					
Extract (CD)	3	973.75	324.58	567.07	<0.001
Nutrient Solution (S)	3	4208.75	1402.91	131.20	0.032
CD × S	9	148.75	16.52	6.68	<0.001
Error	32	79.16	2.47		

Fig. 6: Effects of cow dung extract and nutrient solution on ascorbic acid content in lettuce.
Bars represent means with standard deviation (n=3). Different letters atop the bars indicate significant differences according to Tukey's test at $P \leq 0.05$. Outcome of two-way ANOVA is represented in the table.



	df	*SS*	*MS*	*F*	*P*-Value
Cow dung					
Extract (CD)	3	21107.06	735.69	17.20	<0.001
Nutrient Solution (S)	3	54619.23	206.52	12.96	0.003
CD × S	9	940.76	11.62	0.729	<0.001
Error	32	446.11	13.94		

Fig. 7: Effects of cow dung extract and nutrient solution on β- Carotene content in lettuce.
Bars represent means with standard deviation (n=3). Different letters atop the bars indicate significant differences according to Tukey's test at $P \leq 0.05$. Outcome of two-way ANOVA is represented in the table.

C_4S_3 (253.67 µg/100 g) and C_3S_4 (250.67 µg/100 g) and they are statistically same. However, it was significant that β-carotene content increased in the same treatment with higher yield. Similar findings were also reported earlier by TABATABAEI et al. (2008).

Conclusion

The results showed that the compositional amounts of the various levels of cow dung extract and nutrient solution were closely associated to quality yield as well as lettuce's nitrate content. The combination of CD_3 (150 g of dry cow dung equivalent extract per liter) and S_4 (60 percent of the standard solution) would produce the highest yield in terms of quality, with a focus on significantly less accumulation of nitrate than the limit recommended by the European Union.

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Conflict of interest

No potential conflict of interest was reported by the author.

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