NITRATE CONTROL AND QUALITY IN HYDROPONIC LETTUCE BY USING COW DUNG EXTRACT AND NUTRIENT SOLUTION

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JUNE, 2020

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A *thesis*
submitted to the Faculty of Agriculture
Sher-e-Bangla Agricultural University, Dhaka-1207
in partial fulfillment of the requirements
for the degree of

MASTER OF SCIENCE (MS)
IN
HORTICULTURE

SEMESTER: JANUARY-JUNE, 2020

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*This is to certify that thesis entitled, "**NITRATE CONTROL AND QUALITY IN HYDROPONIC LETTUCE BY USING COW DUNG EXTRACT AND NUTRIENT SOLUTION**" submitted to the faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of **MASTER OF SCIENCE IN HORTICULTURE,** embodies the result of a piece of bona fide research work carried out by **ABU RAIHAN,** Registration:* **12-04806** *under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.*

I further certify that any help or source of information received during the course of this investigation has been duly acknowledged.

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Dedicated to My Beloved Parents

ACKNOWLEDGEMENTS

All praises are due to the ***Almighty Allah****, Who has enabled the author to complete the research work and to prepare this thesis for the degree of Master of Science (M.S.) in Horticulture. The author wishes to express his gratitude and best regards to his respected Supervisor,* ***Prof. Dr. Md. Jahedur Rahman,*** *Department of Horticulture, Sher-e-Bangla Agricultural University, Dhaka, for his continuous direction, constructive criticism, encouragement and valuable suggestions in carrying out the research work and preparation of this thesis.*

The author wishes to express his earnest respect, sincere appreciation and enormous indebtedness to his respected Co-supervisor, ***Prof. Dr. Jasim Uddain****, Department of horticulture, Sher-e-Bangla Agricultural University, Dhaka, for his scholastic supervision, helpful commentary and unvarying inspiration throughout the research work and preparation of the thesis.*

The author feels to express his heartfelt thanks to the honorable Chairman, Prof. Dr. Md. Jahedur Rahman, Department of Horticulture along with all other teachers and staff members of the Department of Horticulture, Sher-e-Bangla Agricultural University, Dhaka, for their co-operation during the period of the study.

The author feels proud to express his deepest and endless gratitude to all of his course mates and friends to cooperate and help his during taking data from the field and preparation of the thesis. The author wishes to extend his special thanks to his lab mates, class mates and friends for their keen help as well as heartiest co-operation and encouragement.

The author expresses his heartfelt thanks to his beloved parents and all other family members for their prayers, encouragement, constant inspiration and moral support for his higher study.

June, 2020 ***The Author***

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ABSTRACT

Hydroponic lettuce, like most leafy greens, can accumulate high levels of nitrates that are often harmful to human health. The accumulation of nitrate closely related to the amount and composition of the nutrition applied. Therefore, to minimize the nitrate content on edible parts of lettuce and to access the production of it, this experiment was conducted by using four different levels of aerated cow dung extracts (C_1 = cow dung extract 50 g.L^{-1}, C_2= cow dung extract 100 g.L^{-1}, C_3 = cow dung extract 150 g.L^{-1}, C_4 = cow dung extract 200 g.L^{-1}) and four modified strength of nutrient solution (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) as a treatments. It was carried out in completely randomized design (CRD) with three replication in semi-green house at Horticulture farm of Sher-e-Bangla Agricultural University, during September 2018 to February 2019. Various growth, physiological and physicochemical parameters of were estimated in this experiment. In case of cow dung extract, the highest leaf length (23.54 cm), maximum number leaves plant^{-1} (17.01) and the highest fresh weight (112.05 g/plant) were recorded from C_3 while the lowest in C_1. For hydroponic nutrient solution, the highest leaf length (23.13 cm), maximum number leaves plant^{-1} (16.66) and the highest fresh weight (116.0 g/plant) were recorded from S_4 while the lowest in S_1. For cow dung extract, the nitrate content (255.48 mg kg^{-1} FW) was statistically higher in C_3, which also had a higher ascorbic acid content (45.41 mg/100 g FW), β-carotene content (239.00 μg/100g) and net assimilation ratio, compared to the lowest in C_1. The content of nitrate (281.24 mg kg^{-1} FW), ascorbic acid (35.41 mg/100 gm FW) and β-carotene (226.17 μg/100g), as well as the net assimilation ratio were the highest for S_4, the lowest for S_1 in the context of nutrient solution. Therefore, it can be concluded that among the treatments combination, C_3S_4 would be much suitable for lettuce production in hydroponic system with highest yield along with far less accumulation of nitrate.

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ABBREVIATIONS AND ACRONYMS

ANOVA	= Analysis of Variance
BCSIR	=Bangladesh Council of scientific and Industrial Research
CV %	Percent Coefficient of Variation
DAT	= Days after transplanting
df	= Degrees of freedom
EC	=Electrical Conductivity
et al.,	= And others
ANOVA	= Analysis of Variance
LA	=Leaf Area
LAR	=Leaf Area Ratio
LDR	= Leaf Dry Weight
LMR	= Leaf Mass Ratio
RWR	= Root Weight Ratio
RGR	= Relative Growth Rate
NAR	= Net Assimilation Ratio
RDR	= Root Dry Weight
Viz.	= namely

CHAPTER I

INTRODUCTION

The significance of high-potential cultivation techniques for growing safe vegetables is becoming increasingly relevant throughout the world. Bangladesh is also giving top priority in modern technology-based agriculture especially agricultural research to enforce food and nutritional protection. In respect to food security, balanced nutrition and income generation, hydroponics is getting increased global attention. As a climate-smart approach, especially by addressing production gaps and inefficiencies in the context of future climate uncertainty, hydroponics for horticultural crops comes first in Bangladesh's agriculture sector. Hydroponics offers a number of benefits, including reuse of water and nutrients, easy environmental control and prevention of soil-borne diseases and pests (Lommen, 2007) and it can also address the salinity problem of a coastal region in countries like Bangladesh. Soilless vegetable cultivation is very important in Bangladesh because there is a scarcity of cultivable land for crops. On the other hand, people of Bangladesh are suffering from many serious health diseases due to lower production and consumption of vegetables. Therefore, hydroponics can be a good alternative to grow sustainably high quality vegetables, like lettuce *(Lactuca sativa L.)* which is one of the most commercial salad vegetables due to its nutritional qualities of vitamins and minerals. Growing lettuce without soil can lead to greater quantity and quality (Sikawa and Yakupitiyage, 2010) but nitrate risk issues are still present. Many plants tend to accumulate nitrate in their leaves, and this is true of lettuce (Gent, 2003). The primary constituent for hydroponic lettuce production is nutrient solution; using high concentrations readily available mineral nutrients, plant grown in this method showed higher levels of nitrate accumulation compared to those grown in conventional systems (Beninni *et al*., 2002). High levels of nitrate in lettuce undesirable because highly accumulated nitrate in plants are harmful to both human and plant growth (Anjana and Iqbal, 2007). Lettuce is actually eaten in natural and as such consumer's concern has been steadily increasing and become an important issue. 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).

Nutrient solution and its constituents can influence the cultivation of quality vegetable crops in hydroponics since these systems allow the manipulation of crop fertilization to modify characteristics such as growth (Oki and Lieth, 2004) and mineral nutrient concentration in plant tissue (Gent, 2003). The most obvious strategy for lowering the nitrate in leafy vegetables is to reduce the availability nitrate to the plant, for example: partial replacement of NO_3-N by other N sources, such as urea or amino acids (Abu-Rayyan *et al*., 2004; Pavlou *et al*., 2007) or limited substitution of NH_4-N to NO_3-N may control nitrate in the internal lettuce leaves (Savvas *et al*., 2003). Ammonium can be used as part of a mixed nitrogen source in hydroponic solutions (Miyata and Ikeda, 2005) when it is not the major source of N and the current recommendation for soilless culture is that NH_4-N should not exceed 25% of the total-N supply (Sonneveld, 2002). Some plants, however, prefer NH_4^+ (Britto and Kronzucker, 2013) such as lettuce, known as ammonium-philic plant (Ikeda and Osawa1981), whereas a few plant roots absorb organic N directly (Näsholm *et al.,* 2009). Therefore, the use of liquid organic fertilizer such as cow dung extract as a partial replacement or supplement 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 and Mahadeen, 2008). Researches have shown that organic fertilizers have improved both the quantity and the quality of lettuce. 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) and many farmers are now turning to organic or "Low Input" farming as a strategy for economic survival. Cow dung is considered as a potential source of good quality organic fertilizer in Bangladesh. 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 micro nutrients besides appreciable quantities of organic matter (Islam, 2006). But the direct use of organic fertilizer proved to be deleterious to plant growth (Garland *et al*., 1997). Therefore, organic fertilizer has been microbially pre-processed before incorporation into hydroponic solutions (Atkin and Nichols, 2004). The pre-processing can generate 25 to 50% is ammonium ($NH4^{+}$) as an intermediate product and nitrate efficiently through ammonification and nitrification of organic fertilizer in the hydroponic solution (Shinohara 2006). However, the use of organic manures alone also cannot fulfill the crop nutrients requirement (Kondapa *et al*., 2009). Bokhtiar *et al.*, 2008 reported that organic manures, when applied with chemical fertilizers gave better yield than individual ones. A reduction in nitrate content can add value to vegetable products which already very popular for their nutritional and therapeutic properties (Santamaria, 2006) and this quality of lettuce can be influenced both by the mineral and organic fertilizer type (Villaga *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 as follows.

1. To find out the appropriate dose of cow dung slurry for maximize the yield and quality of lettuce and
2. To find the effect of cow dung slurry on NO_3^- concentration in lettuce grown in hydroponic system.

CHAPTER II

REVIEW OF LITERATURE

Lettuce is one of the most popular and important salad vegetables, containing calcium, potassium, iron, protein, and fiber is cultivated commercially by hydroponics system throughout the world. Being a succulent leafy vegetable it uptake much more available nitrate and transfer to the leaves. Yet excess nitrate accumulated in the leaves, which is eaten raw is unwanted.

Several human health hazards due to nitrate toxicity have been identified. Some other studies including Gupta *et al*., (2006) and Ustyugova *et al*., (2002) reported that the toxicity of nitrate effects are infant mortality, early onset of hypertension, hypothyroidism, diabetes and an adverse effect on cardiac muscles, alveoli of the lungs and adrenal glands. It also affects the human immune system.

But there have been very few studies in our country on regulating the NO_3^- content and the growth of lettuce in hydroponic systems. However, numerous studies with varying degrees of success have been conducted in different countries of the world for controlling NO_3^- content in lettuce leaves. Some of the research findings related to feasible production and to the optimization of NO_3 concentration in lettuce have been reviewed.

Resh (2012) reported that Hydroponics is a method of growing plants using mineral nutrient solutions in water without soil. It is a technology designed for arid countries like Namibia, where it is advantageous over soil based vegetable production in that it conserves water avoids soil-borne diseases, makes vegetable production possible even in areas with poor soil fertility and generally enhances vegetable production and quality.

Liang *et al*., (2014) reported that traditionally, organic nutrient solution for hydroponics has not been feasible, despite the similarities in plant growth when either conventional or organic fertilizer is applied on soil. It was not until the early 1990s when liquid organic nutrient solutions for hydroponics were introduced. Challenges with these liquid nutrient solutions emerged, such as organic fertilizer being unsuitable to plant growth because nitrogen in organic sources is predominantly organic, hence unusable by plants. The forms of nitrogen absorbed by plants are nitrate and ammonium. Therefore, the nitrogen in manure requires to be mineralized prior to use by plants hydroponically.

Osman *et al*., 2009 reported that the use of mineral fertilizers for agriculture is relatively expensive worldwide and particularly in Africa (Sanchez, 2002). Yet nutrition required for food production (quality and quantity) remains a priority for food security in general, and for vegetables value chains in particular. Farmers therefore, use little or no commercial fertilizer for fear of high cost (Mowa, 2015). Therefore, the current trend depending on expensive fertilizers has failed to achieve the benefit of increased production from use of available critical macro and micro nutrients as a means of increasing the value addition of specialized production for the horticultural market. The goal in retrospect is to search for alternative means for specialized horticultural production.

Gruda (2012) found that different types of materials have long been traditionally used in horticulture and in the nursery industry. Over the last few decades there has been, on the one hand, a significant increase in the number of materials used, arising from industrial processes, to be used with or in replacement of traditional materials and, secondly, there has been a growing use of substrates of cultivation.

Voors *et al.,* (2016) reported that first, adoption of hydroponics technology is practical to local farmers in that it uses simplified local resources such as cow dung manure, goat manures which are abundantly available in the Erongo region as inputs. In contrast to the already failed adoption of hydroponics based on the non-accessible costly resources that come along with the use of hydroponics based on conventional hydroponic fertilizers. Therefore, in the second instance, access to finance for local farmers to participate in hydroponic vegetable production is another handicap for most cannot afford to sustain hydroponic operations based on the current costs associated with conventional hydroponics.

In the third instances, with the abundance of organic sources of nutrients such as goat, cattle and chicken manures in the Erongo region, local community members i.e. those raring goats can form social network groups where they could encourage those within their circles to upscale the local organic hydroponic solution for vegetable production in contrast to the conventional hydroponic nutrient solution which according to the locals is to be afforded by only certain members of society with financial abilities.

Zhou *et al.,* (2000) reported that Nitrate accumulation in vegetables often depends on the amount and kind of nutrients present in the soil and is closely related to the time of application, and the amount and composition of the fertilizers applied.

Inal and Tarakcioglu (2001) found that the use of fertilizers based on ammonia or a mixture of nitrate and ammonium can reduce the nitrate content in plants and it is worthy of further consideration in soilless growth systems because ammonium reduces the need for adding acids to the nutrient solution to lower its pH.

Nitrate accumulation in plants is affected greatly by environmental factors. Santamaria *et al.,* (2001) observed an interaction between light intensity, nitrogen availability and temperature on nitrate accumulation in rocket. Under conditions of low light availability, an increase in temperature increases the nitrate accumulation. Under high light intensity, an increase in temperature increases the nitrate content mainly when the nitrogen supply is high. From this sdudy it also found that nitrate concentration in plants can also be manipulated by stopping the nitrogen supply for some days before crop.

Several studies including Garland *et al.,* (1997) and Shinohara *et al.,* (2011) have since demonstrated that using microorganisms to degrade organic nitrogen in organic sources such as manure results in nitrates and ammonium production which in turn are used for plant production.

Recently, there have been successful hydroponic production of tomato and other vegetables using organic nutrient solutions processed by microorganisms. Chinta *et al.,* (2015) found that using organic nutrient solution made from corn steep liquor not only made successful Lactuca sativa (lettuce) production, but also reduced root rotting.

Fujiwara *et al.*, (2012) found the same effect of reducing root rotting was also observed in tomato plants when organic nutrient solution was used. Furthermore, plant wilting was also reduced in this case. Chinta *et al* (2015) found that using organic nutrient solution made from corn steep liquor provided resistance to air-borne disease in vegetables.

Shinohara *et al.,* (2011) found that using organic nutrient solutions made from fish-based fertiliser or corn steep liquor hydroponically, produced tomato yield similar to those produced from conventional nutrient solutions. From the same organic nutrient solutions, Shinohara *et al.*, (2011) further established that when Lactuca sativa (lettuce) was grown, the organic system produced significantly greater and fresh Lactuca sativa (lettuce) head weight than in the conventional system.

Voogt (2002) indicates that the nutrient solution composition must mediate the uptake ratios of individual elements by the crop and as the demand between distinctive differs, the basic composition of a nutrient solution is specific for each crop. It must also be taken into account that the uptake vary between elements and the system used. For instance, in open-systems with free drainage, much of the nutrient solution is lost by leachate

Bergquist *et al*., (2007) reported that with the exception of carbon (C) and oxygen (O), which are supplied from the atmosphere, the essential elements are obtained from the growth medium. Other elements such as sodium, silicon, vanadium, selenium, cobalt, aluminium and iodine among others, are considered beneficial because some of them can stimulate the growth, or can compensate the toxic effects of other elements, or may replace essential nutrients in a less specific role. The most basic nutrient solutions consider in its composition only nitrogen, phosphorus, potassium, calcium, magnesium and sulphur and they are supplemented with micronutrients. The nutrient composition determines electrical conductivity and osmotic potential of the solution.

Tyson *et al*., (2007) conveyed a study to determine the nitrification rate response in a perlite trickling bio filter (root growth medium) exposed to hydroponic nutrient solution, varying NO_3^- concentrations and two pH levels (6.5 and 8.5), founded that nitrification was significantly impacted by water pH. The increased ammonia oxidation rate (1.75) compared to nitrite oxidation rate (1.3) at pH 8.5 resulted in accumulation of NO_2^- to levels near those detrimental to plants. The potential for increased levels of un-ionized ammonia, which reduced plant nutrient uptake from micronutrient precipitation, are additional problems associated with pH 8.5. Phosphorus is an element which occurs in forms that are strongly dependent on environment pH.

De Rijck and Schrevens (1999) found that each nutrient shows differential responses to changes in pH of the nutrient solution as described below. In the nutrient solution, NH_3 only forms a complex with H^+. For a pH range between 2 and 7, NH_3^+ is completely present as NH_4^{+}. Increasing the pH above 7 the concentration of NH_4^+ decreases, while the concentration of NH_3^+ augments.

Trejo-Téllez *et al*., (2007) reported that with the exception of carbon and oxygen, which are supplied from the atmosphere, the essential elements are obtained from the growth medium. Other elements such as sodium, silicon, vanadium, selenium, cobalt, aluminum and iodine among others, are considered useful because some of them can incite the growth, or can compensate the toxic effects of other elements, or may replace essential nutrients in a less specific role. The most basic nutrient solutions consider in its composition only nitrogen, phosphorus, potassium, calcium, magnesium and sulphur and they are supplemented with micronutrients. The nutrient composition determines electrical conductivity and osmotic potential of the solution.

Samarakoon *et al.*, (2006) conducted that toxicities could occur in nutrient solutions over time, as solution gets concentrated due to rapid water absorption.

Therefore, determination of individual nutrient requirements in different growth stages is needed for the replacement of the nutrient solutions during the growth period. Leaf number of lettuce was not significantly affected by the treatments, since it did not either increase or decrease with increasing nutrient solution concentration.

Samarakoon *et al.*, (2006) reported that leafy lettuce in stationary (trough) culture of hydroponics successfully under tropical greenhouse conditions (38.5ºC). A solution concentration of 0.5 g/L of Albert's solution (having an EC of l.4 dS/m) with renewal at 2 weeks intervals could be identified as the best fertigation strategy under hot and humid conditions. Increasing solution concentrations above that level up to 2 dS/m increased the plant uptake of N, P, K and Ca but, without a significant increase in leaf growth and yield

Dufour and Guérin (2005) carried that when a nutrient solution is used successfully, plants can uptake ions at very low concentrations. So, it has been reported than a high proportion of the nutrients are not used by plants or their uptake does not impact the production. It was determined that in anthodium, 60% of nutrients are lost in the leachate.

Andriolo *et al.*, (2005) found the results whereby leaf number was not affected by salinity levels. Fresh mass decreased with increasing nutrient solution concentration but there was no significant difference between the treatments. This decreases meant that there was a decline in yield of lettuce during the spring season.

Andriolo *et al.*, (2005) conducted that there was no significant difference on root dry mass among treatments because it did not show any distinctive tendency of either increasing or decreasing with increasing nutrient solution concentration. However, there was contrasting results between fresh mass and leaf dry mass whereby fresh mass was decreasing with an increase in nutrient concentration while leaf dry mass was increasing with increasing nutrient concentration. This could be attributed to the fact that plants grown at 1 mS/cm had more water content whereas plants grown a higher EC level (4 mS/cm) had less water content but more dry matter content. This indicate that there was very little nutrients (nutrient deficiency) in the lower EC (1 mS/cm) while high salt content resulted in low chlorophyll content in the higher EC levels (4 mS/cm). Nitrogen significantly increased with increasing nutrient solution concentration. Phosphorus is good for root development but there was conflicting relationship between the P content in the leaves and the dry root mass which could not be explained. Calcium (Ca) decreased with increasing the EC level while magnesium (Mg) remained constant, but both were slightly lower than the recommended range. However, potassium (K) was below the recommended range although it did not affect lettuce quality/taste.

Dufour and Guéri (2005) reported that when a nutrient solution is applied continuously, plants can uptake ions at very low concentrations. So, it has been reported than a high proportion of the nutrients are not used by plants or their uptake does not impact the production. It was determined that in anthurium, 60% of nutrients are lost in the leachate.

Kang and Van Iersel (2004) found that high concentrated nutrient solutions lead to additional nutrient uptake and therefore toxic effects may be expected. Conversely, there are evidences of positive effects of high concentrations of nutrient solution. In salvia, the increase of Hoagland concentration at 200% caused that plants flowered 8 days previous to the plants at low concentrations, increasing total dry weight and leaf area.

Voogt (2002) indicates that the nutrient solution composition must mediate the uptake ratios of individual elements by the crop and as the demand between distinctive differs, the basic composition of a nutrient solution is specific for each crop. It must also be taken into account that the uptake vary between elements and the system used. For instance, in open-systems with free drainage, much of the nutrient solution is lost by leachate.

Serio *et al*., (2001) found decreasing fresh shoot mass with increasing nutrient solution concentration.

Samarakoon *et al.*, (2006) reported that the EC values for hydroponic systems range from 1.5 to 2.5 ds m-1. Higher EC hinders nutrient uptake by increasing osmotic pressure, whereas lower EC may severely affect plant health and yield.

Mowa (2015) found that when goat manure was used hydroponically after processing in compost piles, Beta vulgaris subspecies cicla var. flavescens (spinach) production was made possible through production was less than conventional hydroponics.

Materska *et al.,* (2005) reported that there was no significant difference on root dry mass among treatments because it did not show any specific tendency of either increasing or decreasing with increasing nutrient solution concentration. However, there was contrasting results between fresh mass and leaf dry mass whereby fresh mass was decreasing with an increase in nutrient concentration while leaf dry mass was increasing with increasing nutrient concentration. This could be attributed to the fact that plants grown at 1 mS/cm had more water content whereas plants grown a higher EC level (4 mS/cm) had less water content but more dry matter content.

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Sarro *et al.,* (2007) found decreasing fresh shoot mass with increasing nutrient solution concentration in hydroponic system.

Salam (2001) showed that nitrogen enhances the protein synthesis, which allows plant to grow faster, rate of metabolism, cell division, cell elongation and thereby stimulated apical growth.

CHAPTER III

MATERIALS AND METHODS

3.1 Experimental Site

The experiment was replicated twice from September 2018 to March 2019. It was conducted in the semi-greenhouse at the Horticulture Farm, Sher-e- Bangla Agricultural University, Dhaka 1207, Bangladesh. The site of study is located at latitude 23°74/ N and longitude 90°35/ E.

3.2 Experimental arrangement

The present researches were conducted in structure using polyvinyl chloride (PVC) pipes. The structure consisted of four 5- feet growing tubes/beds made of 5" PVC pipe and a stand and trellis made up with strong and durable steel. The stand measuring 3 feet x 3 feet x 2.5 feet where the four growing tubes were installed and each pipe has been considered as an experimental unit. Holes were made on the upper part of the pipe with the help of drill machine and the distance between two holes was 19.06 cm.

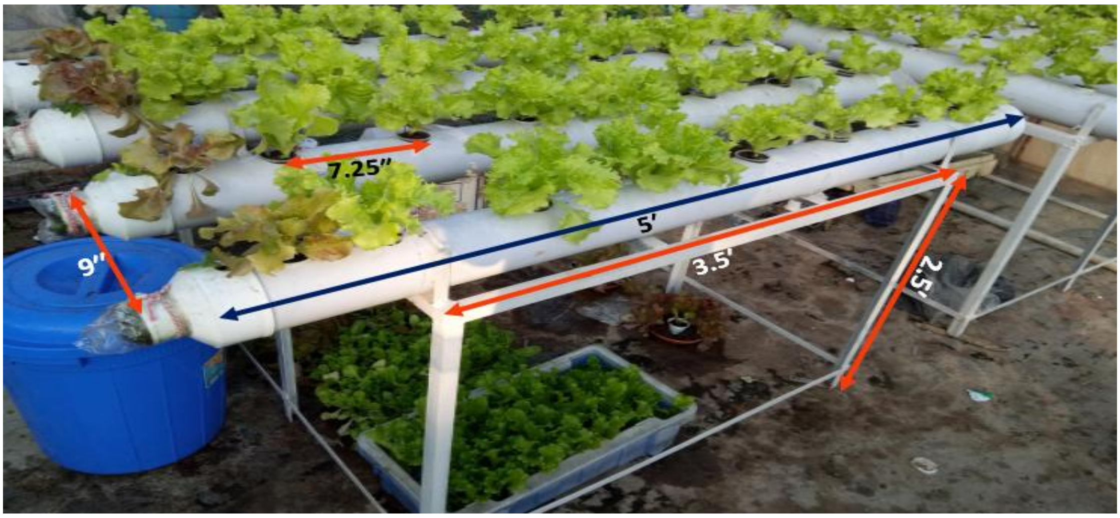

Plate 1. Growing lettuce plants on a hydroponic structure

Pipes had been placed horizontally on this stand, as the holding plants become more exposed to sunlight. Every pipe accommodated up to 8 plants.

3.3 Experimental design and treatment:

The two factors experiment were conducted in a completely randomized design (CRD) with three replications. The two factors were as follows:

Factor A: Four different types of cow dung extract denoted as CD:

CD_1 = Cow dung extract 50 $g.L^{-1}$,

CD_2= Cow dung extract 100 $g.L^{-1}$,

CD_3 Cow dung extract 150 $g.L^{-1}$, and

CD_4 = Cow dung extract 200 $g.L^{-1}$

Factor B: Four different modified strength nutrient solution Rahman and Inden (2012) denoted as S:

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

3.4 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 basic nutrient solution which was used in treatments was Rahman and Inden (2012) solution. The nutrient solution was prepared with distilled water and chemical grade reagents. The ratio of Rahman and Inden (2012) solution were NO3-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). and (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 sources of microbial inoculum.

3.4.1 Collection of cow dung:

Cow dung was collected from SAU animal farm in the, where the manure was stored for few days in an open storage. Cow dung was chosen because it is abundantly available due to dominance of small stock farming in our country.

3.4.2 Preparation of cow dung extract:

The cow dung manure was homogenized, air-dried for 1 week and ground in a chamber treated by Mazim which was contained in plastic cans and incubated at ambient temperature before use it. In order to determine levels of mineral nutrients in the treated cow dung manure before formulating the nutrient solution, it was immersed and fermented in groundwater for 24 hours in ratios of 1:10 accordance with (Charoenpakdee, 2014)}. The mixture was stirred every 7 days during fermentation process. Then cow dung slurry was aerated by manual agitation twice a day for 21 days. After that it was filtered through a thin white cloth and diluted a solution with groundwater in ratios of 1:3 and used as solution additives.

3.4.3 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).

3.5 Seed sowing and Planting

Seeds of a loose leaf type lettuce (*Lactuca sativa* cv. 'Green Wave') were collected from Siddik Bazar, Dhaka, in a sealed package and germinated in seedling trays. Each cell of tray was filled by a hydroponic planting basket (sauce cup) loaded with germinating media, consist of 1/5 extract byproduct, 1/5 khoa, 1/3 coconut coir and 1/3 sawdust (v/v). Trays were kept on applying mist until plants had two true leaves when they were moved to a greenhouse with natural day/night light conditions and an average temperature of 25 °C. All plants were watering with ¼th strength Rahman and Inden (2012) solution from the two true leaves stage, until seedlings were ready for transplantation. Four weeks after sowing, potted seedlings along with the same supporting media that used for seed germination were transplanted to the hydroponics systems at a spacing of 22×18cm. The respective treatment solutions had been filled with the individual pipes that can hold up to 16 liters of solution at controlling level. In these pipe channels the plant's roots grew freely on the growing media.

3.6 Interculture operation

3.6.1 Weeding

No weeding was done in the experiment.

3.6.2 Insect management

Lettuce plants were grown in controlled environment. So, no insecticides were applied in the experiment

3.6.3 Diseases management

Lettuce plants were grown in controlled environment in hydroponic system and all nutrients required for plant were supplied artificially to the plants. The growing environment was clean and no disease attacked to the plants.

3.7 Harvesting and measurement

The crop was harvested after 42 days of sowing. This loose leaf variety lettuce can be cut from the stem when it is young and for a fresh for a delightful salad. At random three plants from each treatment were harvested and washed with tap water. Substrates in the roots of plants from substrate 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. After assessing the leaf area, the leaves and roots were put separately in paper bags. Total yields were also recorded.

Plate 2. Harvesting of lettuce for data collection

3.8 Data collection

Different data on the growth and physiological traits were recorded during the experiment. Data were collected from each plant described below.

3.8.1 Plant growth parameter

Growth components e.g. plant height, leaf breadth, leaf length, per plant leaves number fresh weight and dry matter of plant was assessed to study morphological traits among treatments.

3.8.1.1 Plant height

Plant height was measured in centimeter (cm) by a meter scale at 0, 7, 21, 28, 35 and 42 DAT (days after transplanting) from the point of attachment of supporting media up to the tip of the longest leaf.

3.8.1.2 Breadth of leaves

Breadth of leaves were measured in centimeter (cm) by a meter scale at 0, 7, 21, 28, 35, and 42 DAT. Fourth leaf was selected for measurement of breadth.

3.8.1.3 Length of leaves

Length of leaves were measured in centimeter (cm) by a meter scale at 0,7, 21, 28, 35, and 42 DAT. Fourth leaf was selected for measurement of length.

3.8.1.4 Number of leaves per plant

Number of leaves per plant were counted at 0, 7, 21, 28, 35, and 42 DAT. All the leaves of each plant were counted separately. Only the smallest young leaves at the growing point of the plant were excluded from the counting and the average number was recorded.

3.8.1.5 Fresh weight of plant

Leaves were detached by a sharp knife and fresh weight (g) of the plant was taken by an electric balance at harvest (42 DAT) and was recorded.

3.8.1.6 Percent dry matter of plant

The random samples of plants were sun dried for seven days then oven-dried at 70 ° C for 72 h. After drying, plants were weighed for constant dry weight on a scale accurate to 0.0001g. The dry matter of plant was estimated on percentage basis. The percentage of plant dry matter was calculated by using the following formula.

$$\% \text{ Dry matter of plant} = \frac{Constant\ dry\ weight\ of\ plant}{Fresh\ weight\ of\ plant} \times 100$$

3.8.2 Physiological traits

Different physiological parameters [Leaf area (LA), leaf area ratio (LAR), leaf mass ratio (LMR), root weight ratio (RWR), relative growth rate (RGR), and net assimilation rate (NAR)] were determined in the experiments. The parameters were measured as described

$$LAR = \frac{LA}{PDW} \text{ -- } (1),$$

Where LAR = leaf area ratio, LA = Leaf area (cm2), PDW = plant dry weight (g).

$$LMR = \frac{LDW}{PDW} \text{ -- } (2),$$

where LMR = leaf mass ratio, LDW = leaf dry weight (g).

$$RWR = \frac{RDW}{PDW} \text{ --- } (3),$$

where RWR = root weight ratio, RDW = root dry weight (g).

$$RGR = \frac{(PDW1 - PDW0)}{(t1 - t0)} \times PDW_0 \text{ ----------------------------- } (4),$$

where t = time, subscripts 0 and 1 refer to the transplanting and final harvest (days), respectively.

$$NAR = \frac{RGR}{LAR} \text{ -- } (5)$$

3.8.3 Estimation of β- Carotene, ascorbic acid and NO_3 content:

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).

3.8.4 Determination of SPAD value:

Since chlorophyll mostly consisted of nitrogen containing enzymes and other organic compounds, there is a positive correlation between the nitrogen and chlorophyll concentrations of leaves. 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.

Plate 3. Determination of SPAD value by SPAD plus chlorophyll meter

CHAPTER IV

RESULTS AND DISCUSSION

The results of the experiment conducted under semi greenhouse conditions were presented in table 1 to table 9 and figure 1 to figure 2. The experiment was conducted to determine the production of lettuce and the accumulation of nitrate in it as influenced by organic substrates. The results were presented and discussed under the following sub heading.

4.1 Vegetative growth and yield parameters

4.1.1 Plant height

There were significant differences in plant height at 7, 14, 21, 28 and 42 days after transplanting (DAT) in respect of diffident treatments of cow dung extract (Figure 1) and nutrient solution (Figure 2). At 7 DAT, the tallest plant (7.54 cm) was found in CD_4 and the shortest (5.2 cm) was found in CD_1. At 14 DAT, the tallest plant (11.27 cm) was found in CD_3 and the shortest (7.90 cm) was found in CD_1. At 21 DAT, the tallest plant (15.30 cm) was found in CD_3 and the shortest (11.27 cm) was found in CD_1. At 28 DAT, the tallest plant (17.94 cm) was found in CD_3 and the shortest (14.10 cm) was found in CD_1. At 35 DAT, the tallest plant (21.38 cm) was found in CD_3 and the shortest (16.52 cm) was found in CD_1. At 42 DAT, the tallest plant (23.54 cm) was found in CD_3 and the shortest (18.57 cm) was found in CD_1. The results revealed that the plant heights increased in the advancement of plant maturity. It also noticed that and plant height increased in increasing dose of cow dung extract until a certain dose. It might be due to higher amount of nitrogen in cow dung extract. Nitrogen slowly released from cow dung extract might have encouraged more vegetative growth of the plant at later stage of growth. Scientists have reported that different levels of organic manure significantly increased plant height (Yadav and Malik, 2005).

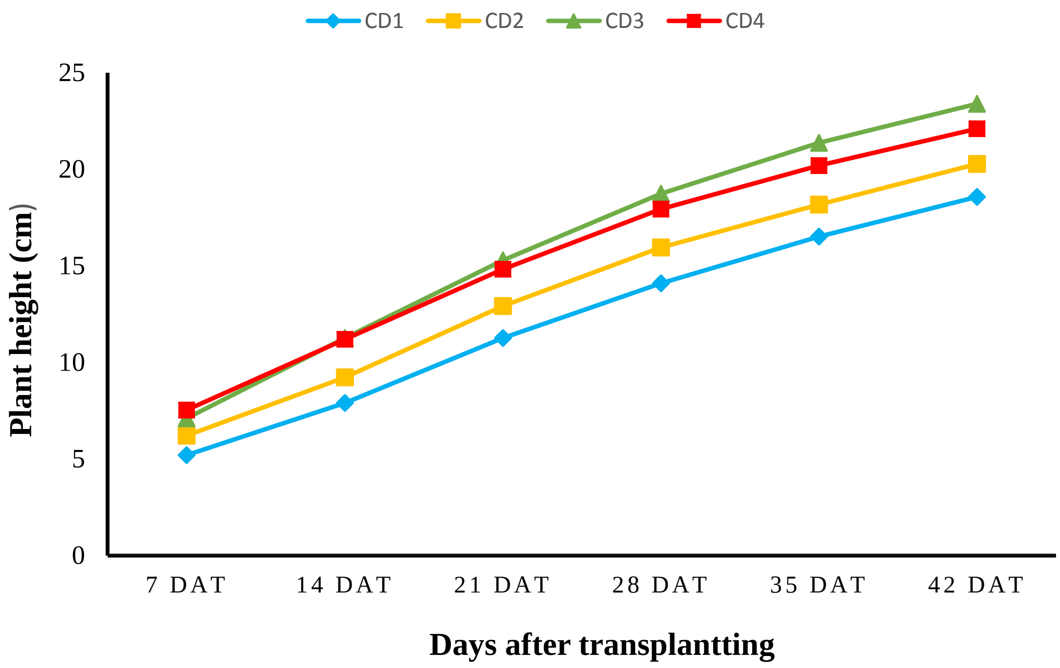

Figure 1. Main effects of cow dung extract on plant height at different days after transplanting (DAT)

Here, CD_1 = 50 g dry cow dung equivalent extract per liter, CD_2= 100 g dry cow dung equivalent extract per liter, CD_3 = 150 g dry cow dung equivalent extract per liter, and CD_4 = 200 g dry cow dung equivalent extract per liter. Days after transplanting (DAT)

In case of nutrient solution, the tallest plant (6.55 cm) was found in S_4 and the shortest (6.48 cm) was found in S_1 which were statistically similar to that of S_3 and S_2 respectively at 7 DAT. At 14 DAT, the tallest plant (10.89 cm) was found in S_4 and the shortest (8.43 cm) was found in S_1. At 21 DAT, the tallest plant (15.20 cm) was found in S_4 and the shortest (11.15 cm) was found in S_1. At 28 DAT, the tallest plant (18.54 cm) was found in S_4 and the shortest (13.67 cm) was found in S_1. At 35 DAT, the tallest plant (21.15 cm) was found in S_4 and the shortest (15.85 cm) was found in S_1. At 42 DAT, the tallest plant (23.13 cm) was found in S_4 and the shortest (17.85 cm) was found in S_1. The results revealed that shortest plants at all dates were found in the plants grown in S_1 and the tallest plant was found in S_4 in all the cases. These might be due to balanced nutrition during the growth period of lettuce and readily availability nitrogen for growth and development, resulting the tallest plant. Similar results were observed by Tittonell *et al*. (2003) and Boroujerdnia and Ansari (2007).

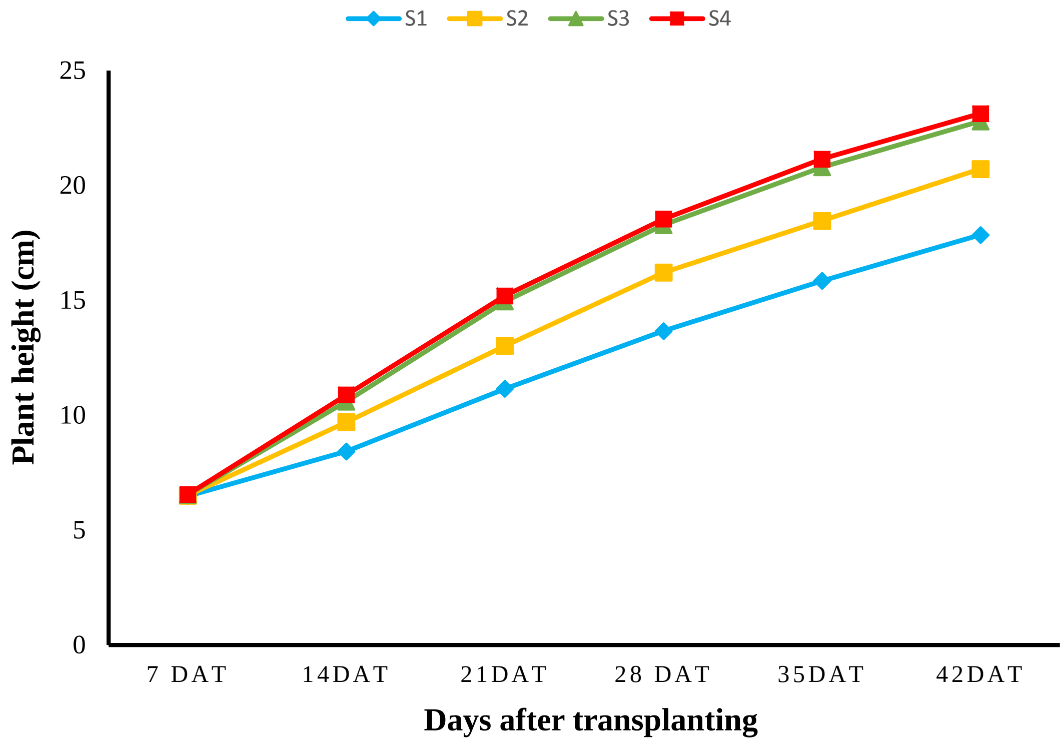

Figure 2. Main effects of nutrient solution on plant height at different days after transplanting (DAT)

Here, S_1 = 30% of the standard solution, S_2 = 40% of the standard solution, S_3 = 50% of the standard solution and S_4 = 60% of the standard solution. Days after transplanting (DAT)

Meanwhile, for the combination of cow dung extract and nutrient solution, plant height of lettuce significantly varied at 7, 14, 21, 28, 35 and 42 DAT (Table 1). At 7 DAT, CD_4S_4 performed the tallest (7.57 cm)height but another of all observation (14 DAT, 12.45 cm; 21 DAT, 17.4 cm; 28 DAT, 21.27 cm; 35 DAT, 24.26 cm and 42 DAT, 26.37 cm) the CD_3S_4 gave the tallest plant and CD_1S_1 showed the shortest plant in all observations. Data presented in Table 1 indicated that cow dung extract and nutrient solution alone and their interaction significantly influenced plant height.

Table 1. Combined effect of cow dung extract and nutrient solution on plant height of lettuce at different days after transplanting

Treatments	Plant height at different days after transplanting(DAT) (cm)					
	7DAT	**14DAT**	**21DAT**	**28DAT**	**35DAT**	**42DAT**
CD_1S_1	5.15 d[z]	6.78 l	9.50 l	11.75 p	14.26 o	16.38 n
CD_1S_2	5.17 d	7.60 k	11.04 k	14.03 n	16.04 m	18.03 l
CD_1S_3	5.23 d	8.42 i	12.03 j	15.03 k	17.52 j	19.58 i
CD_1S_4	5.24 d	8.83 h	12.52 i	15.47 i	18.25 i	20.27 g
CD_2S_1	6.16 c	8.17 j	11.07 k	13.77 o	15.87 n	17.77 m
CD_2S_2	6.18 c	9.18 g	12.51 i	15.27 j	17.53 j	20.03 h
CD_2S_3	6.25 c	9.57 f	13.77 h	17.06 h	19.27 h	21.20 f
CD_2S_4	6.26 c	10.03 e	14.35 f	17.68 f	20.05 f	22.15 e
CD_3S_1	7.08 b	9.27 g	12.01 j	14.67 l	16.77 k	18.77 j
CD_3S_2	7.08 b	11.01 d	14.51 e	18.02 e	20.48 e	22.77 d
CD_3S_3	7.13 b	12.37 ab	17.27 b	21.02 b	24.02 b	26.27 a
CD_3S_4	7.14 b	12.45 a	17.4 a	21.27 a	24.26a	26.37 a
CD_4S_1	7.52 a	9.52 f	12.02 j	14.48 m	16.50 l	18.46 k
CD_4S_2	7.54 a	11.01 d	14.02 g	17.52 g	19.79 g	22.03 e
CD_4S_3	7.54 a	12.02 c	16.77 c	20.02 c	22.37 c	24.17 b
CD_4S_4	7.57 a	12.26 b	16.51 d	19.75 d	22.03 d	23.74c
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[z]Means with different letter is significantly different by Tukey's test at $P \leq 0.05$. CD_1 = 50 g dry cow dung equivalent extract per liter, CD_2= 100 g dry cow dung equivalent extract per liter, CD_3 = 150 g dry cow dung equivalent extract per liter, and CD_4 = 200 g dry cow dung equivalent extract per liter.S_1 = 30% of the standard solution, S_2 = 40% of the standard solution, S_3 = 50% of the standard solution and S_4 = 60% of the standard solution. *P* represents the level of significance of ANOVA.

4.1.2 Number of leaf

Significant variation was recorded for number of leaves/plant of lettuce at 7, 14, 21, 28 35 and 42 days after transplanting (DAT) with application of different levels of cow dung extract (Figure 3) and nutrient solution (Figure 4). At 7 DAT, the maximum number of leaves/plant (5.64) was counted in CD_4 and the minimum number of leaves/plant (4.87) was found in CD_1. At 14 DAT, the maximum number of leaves/plant (7.23) was found in CD_4 and the minimum number of leaves/plant (6.72) was found in CD_1. At 21 DAT, the maximum number of leaves/plant was found in (9.82) CD_3 which was statistically similar that of CD_4 (9.81) and the minimum number of leaves/plant (8.79) was found in CD_1. At 28 DAT, the maximum number of leaves/plant (12.43) was found in CD_3 and the minimum number of leaves/plant (11.04) was found in CD_1. At 35 DAT, the maximum number of leaves/plant (14.68) was found in CD_3 and the minimum number of leaves/plant (12.91) was found in CD_1. At 42 DAT, the maximum number of leaves/plant (16.52) was found in CD_3 and the minimum number of leaves/plant (14.34) was found in CD_1. The results revealed that with the increase of amount of cow dung extract until a certain dose the number of leaves also increased. The increase in number of leaves/plant might be due to availability of nutrients in cow dung extract applied plant during the growth period of the crop and its role in enhancing physical properties of growing environment. Similarly higher number of leaves plant^{-1} was also obtained with cattle manure (Michael et al., 2012).

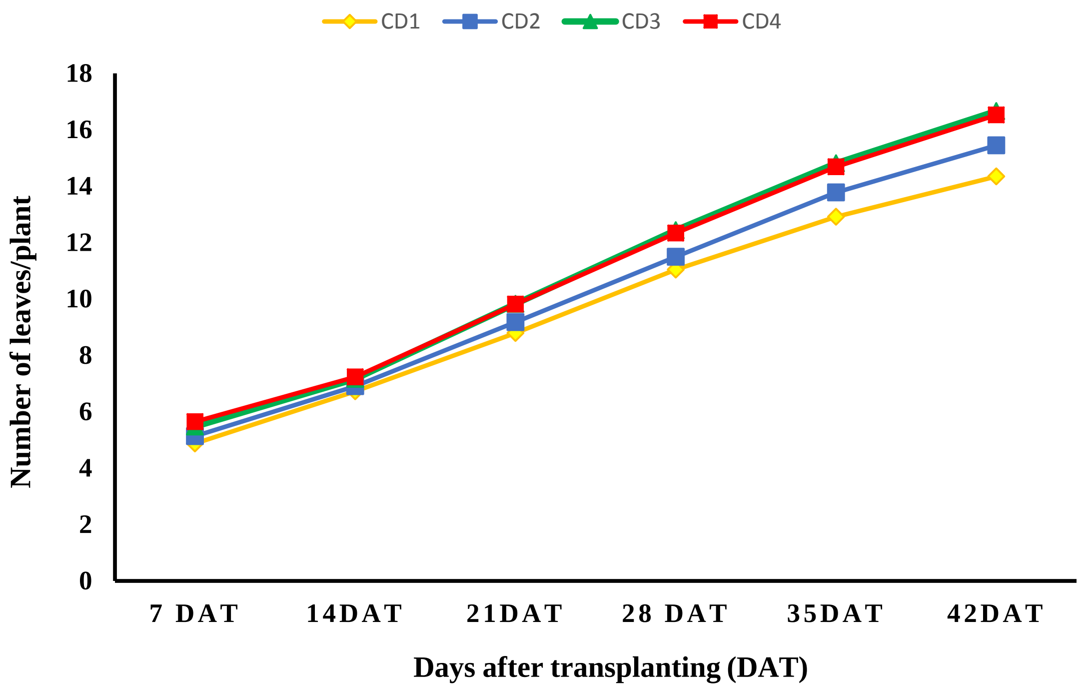

Figure 3. Main effects of cow dung extract on number of leaves of lettuce at different days after transplanting

Here, CD_1 = 50 g dry cow dung equivalent extract per liter, CD_2= 100 g dry cow dung equivalent extract per liter, CD_3 = 150 g dry cow dung equivalent extract per liter, and CD_4 = 200 g dry cow dung equivalent extract per liter. Days after transplanting (DAT)

In case of nutrient solution, the maximum number of leaves/plant (5.29) was found in S_4 which is statistically similar that of S_3 (5.29) and the minimum number of leaves/plant was found in S_1 (5.27) which was statistically similar to that of S_2 (5.28) and S_3 at 7 DAT. At 14 DAT, the maximum number of leaves/plant (7.25) was found in S_4 and the minimum number of leaves/plant (6.71) was found in S_1. At 21 DAT, the maximum number of leaves/plant (9.89) was found in S_4 and the minimum number of leaves/plant (8.71) was found in S_1. At 28 DAT, the maximum number of leaves/plant (12.45) was found in S_4 and the minimum number of leaves/plant (10.85) was found in S_1. At 35 DAT, the maximum number of leaves/plant (15.08) was found in S_4 and the minimum number of leaves/plant (12.43) was found in S_1. At 42 DAT, the maximum number of leaves/plant (17.01) was found in S_4 and the minimum number of leaves/plant (13.41) was found in S_1. The results revealed that the minimum number of leaves/plant at all dates were found in the plants grown in S_1 and the maximum number of leaves/plant was found in S_4 in all the cases. These might be due to favorable condition and availability of more nutrients for plant.

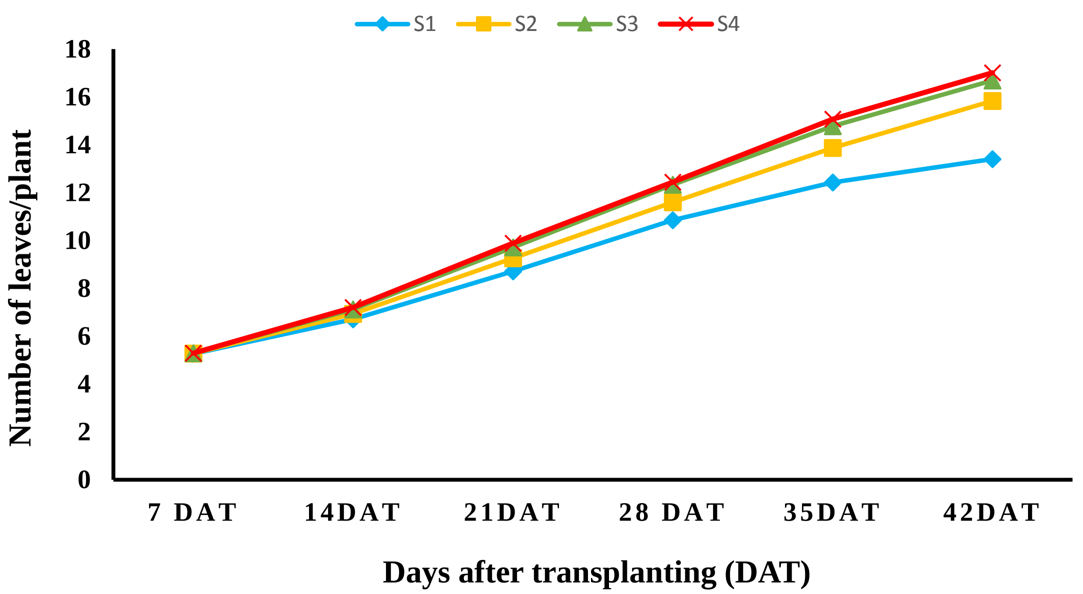

Figure 4. Main effects of nutrient solution on no. of leaf of lettuce at different days after transplanting

Here, S_1 = 30% of the standard solution, S_2 = 40% of the standard solution, S_3 = 50% of the standard solution and S_4 = 60% of the standard solution. Days after transplanting (DAT)

Meanwhile in case of combined interaction of cow dung extract and nutrient solution, significant variation was observed at 7, 14, 21, 28, 35 and 42 DAT (Table 2) in terms of number of leaf of lettuce.

Table 2. Combined effect of cow dung extract and nutrient solution on leaf number of lettuce at different days after transplanting

Treatments	Number of leaf at different days after transplanting(DAT) (cm)					
	7 DAT	14 DAT	21 DAT	28 DAT	35 DAT	42 DAT
CD_1S_1	4.85 d[z]	6.36 i	8.08 i	10.05 n	11.29 n	12.13 n
CD_1S_2	4.86 d	6.65 g	8.67 h	10.90 l	12.78 l	14.44 j
CD_1S_3	4.88 d	6.89 f	9.03 g	11.43 i	13.52 i	15.03 i
CD_1S_4	4.88 d	7.01 e	9.38 e	11.76 h	14.03 g	15.76 g
CD_2S_1	5.13 c	6.58 h	8.62 h	10.76 m	12.07 m	13.04 m
CD_2S_2	5.13 c	6.84 f	9.05 g	11.39 ij	13.77 h	15.57 h
CD_2S_3	5.13 c	7.01 e	9.43 e	11.84 g	14.25 f	16.30 f
CD_2S_4	5.14 c	7.17 c	9.58 d	11.97 f	15.02 d	16.88 d
CD_3S_1	5.46 b	6.88 f	9.03 g	11.25 k	13.29 j	14.35 k
CD_3S_2	5.47 b	7.09 d	9.64 cd	12.18 e	14.49 e	16.67 e
CD_3S_3	5.48 b	7.28 b	10.24 b	13.09 b	15.70 ab	17.77 ab
CD_3S_4	5.48 b	7.42 a	10.36 a	13.21 a	15.76 a	17.83 a
CD_4S_1	5.63 a	7.02 e	9.13 f	11.33 j	13.07 k	14.12 l
CD_4S_2	5.64 a	7.17 c	9.69 c	12.12 e	14.45 e	16.70 e
CD_4S_3	5.65 a	7.32 b	10.19 b	12.99 c	15.66 b	17.70 b
CD_4S_4	5.65 a	7.42 a	10.23 b	12.86 d	15.53 c	17.57 e
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[z]Means with different letter is significantly different by Tukey's test at $P \leq 0.05$. CD_1 = 50 g dry cow dung equivalent extract per liter, CD_2= 100 g dry cow dung equivalent extract per liter, CD_3 = 150 g dry cow dung equivalent extract per liter, and CD_4 = 200 g dry cow dung equivalent extract per liter.S_1 = 30% of the standard solution, S_2 = 40% of the standard solution, S_3 = 50% of the standard solution and S_4 = 60% of the standard solution. *P* represents the level of significance of ANOVA

At 7 DAT, the maximum number of leaves/plant (5.65) was found in CD_4S_4 and CD_4S_3 witch were statistically similar to CD_4S_1 and CD_4S_2, whereas the minimum number of leaves/plant (4.85) was found in CD_1S_1. At 14 DAT, the minimum number of leaves/plant observed in CD_1S_1 (6.36). The maximum number of leaves/plant for CD_3S_4 (7.42) and CD_4S_4 (7.42) which were statistically similar. But at 21, 28, 35 and 42 DAT it was found that the maximum number of leaves/plant number of leaf in CD_3S_4. The pH and EC with other properties were more favorable and ensured appropriate condition for the elongation of lettuce plant with optimum vegetative growth and the ultimate results was the maximum number of leaves/plant in CD_3S_4.

4.1.3 Leaf breath

Leaf breath of lettuce was significantly influenced by diffident treatments of cow dung (Figure 5) at 7, 14, 21, 28, 35 and 42 days after transplanting (DAT). At 7 DAT, the widest leaf breath (3.07 cm) was found in CD_4 and the narrowest (2.16 cm) was found in CD_1. The widest leaf breath 4.89 cm) was found in CD_4 and the narrowest (3.86 cm) was found in CD_1 at 14 DAT. At 21 DAT, the broad leaf (7.04 cm) was found in CD_4 and the small leaf (5.81 cm) was found in CD_1. At 28 DAT, the extensive leaf breath (9.40 cm) was found in CD_4 and the narrowest (7.80 cm) was found in CD_1. At 35 DAT, the widest leaf breath (11.40 cm) was found in CD_3 witch was statistically similar that of CD_4 (11.38 cm) and the narrowest (9.59 cm) was found in M3. At 42 DAT, the widest leaf breath (13.32 cm) was found in CD_4 and the narrowest (11.18 cm) was found in CD_1.The results revealed that the lowest leaf breath at all dates were observed in the plants grown in CD_1 and the maximum leaf breath at all dates were found in plants grown in CD_4. Optimum vegetative growth was occurred due to enhanced amount of nitrogen fertilizer that leads for the growth of lettuce and the ultimate results was the widest leaf. The findings previously obtained by Boroujerdnia and Ansari (2007) were comparable to the present analysis.

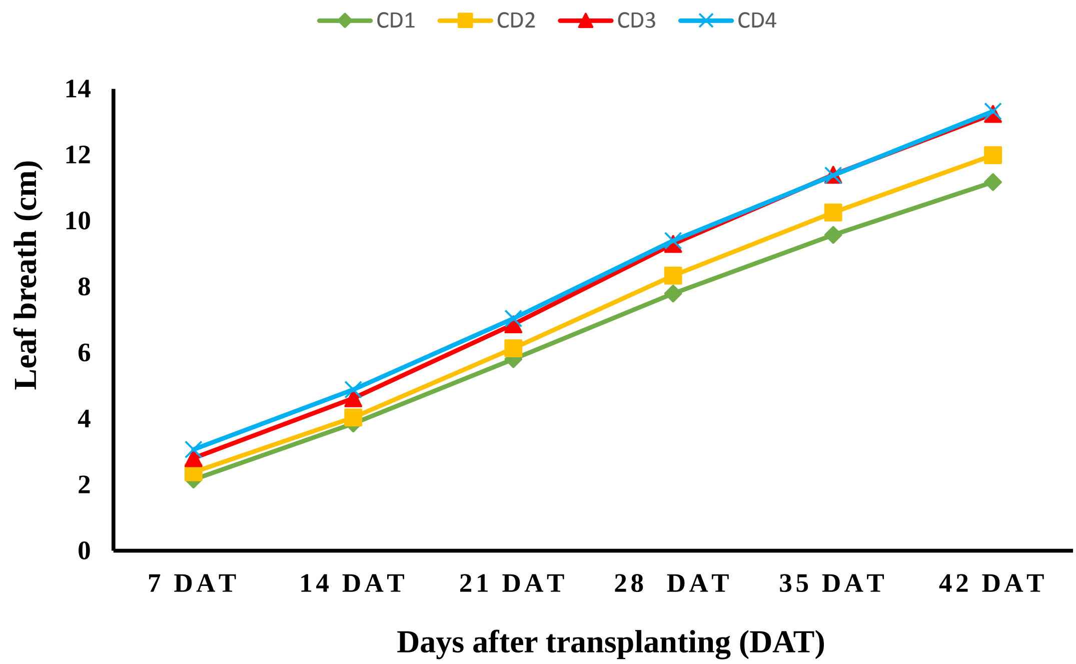

Figure 5. Main effects of cow dung extract on leaf breathe of lettuce at different days after transplanting

Here, CD_1 = 50 g dry cow dung equivalent extract per liter, CD_2= 100 g dry cow dung equivalent extract per liter, CD_3 = 150 g dry cow dung equivalent extract per liter, and CD_4 = 200 g dry cow dung equivalent extract per liter. Days after transplanting (DAT).

Leaf breath differed significantly among the four treatments of nutrient solution at different DAT (Figure 6). The widest leaf breath at 7 DAT (2.62 cm) was found in S_4 witch is statistically similar that of S_3 (2.61) and the narrowest (2.60 cm) was found in S_1. The widest leaf breath (4.73 cm) was found in S_4 and the narrowest (3.86 cm) was found in S_1 at 14 DAT. At 21 DAT, the broad leaf (7.23 cm) was found in S_4 and the small leaf (5.46 cm) was found in S_1. At 28 DAT, the widest leaf breath (9.83 cm) was found in S_4 and the narrowest (7.10 cm) was found in S_1. At 35 DAT, the broadest leaf breath (12.07 cm) was found in S_4 and the narrowest (8.49 cm) was found in S_1. At 42 DAT, the widest leaf breath (14.17 cm) was found in S_4 and the narrowest (9.72 cm) was found in S_1. The results revealed that the maximum leaf breath at all dates were found in plants grown in S_4 and the lowest leaf breath at all dates were observed in the plants grown in S_1.

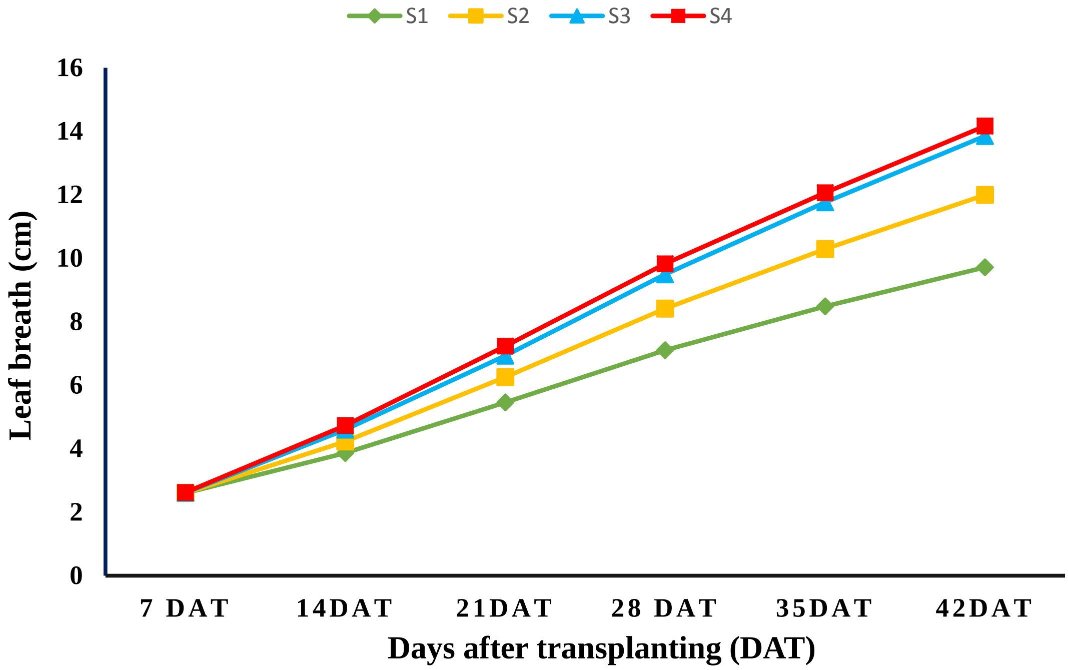

Figure 6. Main effects of nutrient solution on leaf breathe of lettuce at different days after transplanting

Here, S_1 = 30% of the standard solution, S_2 = 40% of the standard solution, S_3 = 50% of the standard solution and S_4 = 60% of the standard solution. Days after transplanting (DAT)

In case of combined effect of cow dung extract and nutrient solution, the significant variation was found at 7, 14, 21, 28, 35 and 42 DAT (Table 3) in terms of leaf breath of lettuce. At 7 DAT, the broadest leaf (3.08 cm) was found in CD_4S_4 and the narrowest leaf breath (2.16 cm) was found in CD_1S_1. At 14 DAT, the narrowest leaf breath observed in CD_1S_1 (3.21 cm) and the widest for CD_4S_3 (5.13 cm) and CD_4S_4 (5.10 cm) which were statistically similar. But at 21 and 28 DAT it was found that the maximum leaf breath in CD_3S_4 (7.54 cm) and CD_4S_4 (10.23 cm). At 35 and 42 DAT it was found that the widest leaf breath in CD_3S_4 (12.52 cm) and CD_3S_3 (14.68 cm). The widest leaf breath almost all dates were found in CD_3S_4 and lowest were found in CD_1S_1.

Table 3. Combined effect of cow dung extract and nutrient solution on leaf breath of lettuce at different days after transplanting

Treatments	Leaf breath at different days after transplanting(DAT) (cm)					
	7DAT	14DAT	21DAT	28DAT	35DAT	42DAT
CD_1S_1	2.16 d[z]	3.21 k	4.54 l	5.88 k	6.99 m	7.93 m
CD_1S_2	2.17 d	3.60 j	5.18 j	7.14 i	8.75 k	10.17 k
CD_1S_3	2.16 d	4.21 g	6.45 g	8.80 f	10.92 g	12.86 g
CD_1S_4	2.17 d	4.44 e	6.92 d	9.39 d	11.66 e	13.75 d
CD_2S_1	2.38 c	3.61 j	5.14 k	6.71 j	8.06 l	9.22 l
CD_2S_2	2.38 c	3.94 i	5.89 i	7.99 g	9.78 h	11.38 h
CD_2S_3	2.38 c	4.21 g	6.61 f	9.11 e	11.36 f	13.42 e
CD_2S_4	2.39 c	4.36 f	6.94 d	9.54 c	11.84 d	13.95 c
CD_3S_1	2.80 b	4.15 h	5.90 i	7.77 h	9.34 j	10.72 j
CD_3S_2	2.81 b	4.54 d	6.76 e	9.18 e	11.29 f	13.22 f
CD_3S_3	2.82 b	4.81 c	7.29 b	10.00 b	12.44 ab	14.65 a
CD_3S_4	2.82 b	5.01 b	7.54 a	10.23 a	12.52 a	14.68 a
CD_4S_1	3.05 a	4.49 de	6.23 h	8.05 g	9.56 i	11.01 i
CD_4S_2	3.06 a	4.84 c	7.06 c	9.38 d	11.34 f	1324 f
CD_4S_3	3.07 a	5.13 a	7.37 b	10.05 b	12.34 bc	14.45 b
CD_4S_4	3.08 a	5.10 a	7.51 a	10.12 ab	12.27 c	14.29 b
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[z]Means with different letter is significantly different by Tukey's test at $P \leq 0.05$. CD_1 = 50 g dry cow dung equivalent extract per liter, CD_2= 100 g dry cow dung equivalent extract per liter, CD_3 = 150 g dry cow dung equivalent extract per liter, and CD_4 = 200 g dry cow dung equivalent extract per liter.S_1 = 30% of the standard solution, S_2 = 40% of the standard solution, S_3 = 50% of the standard solution and S_4 = 60% of the standard solution. *P* represents the level of significance of ANOVA

4.1.4 Fresh weight of lettuce

Marketable lettuce quality is determined primarily by the size of the plant and its fresh weight. Insignificant difference of fresh weight at transplanting time but differed at harvesting among the treatments (Table 4). The total fresh weight increased in increasing days until maturity. At harvest time, for cow dung extract total fresh weight was found higher in CD_3 (112.84g / plant) the lowest fresh weight found in CD_1 (85.19 g / plant) and for nutrient solution it was found to be higher in S_4 (117.63 g / plant) the lowest fresh weight found in S_1 (80.91 g / plant). Higher fresh weight of leaf was found (71.02 g/plant) in CD_3 and the lowest was in CD_1 (53.63 g/plant) in response to cow dung extract and for the nutrient solution the highest and lowest weight found in S_4 (75.73 g/plant) and S_1 (46.99 g/plant) respectively. Fresh weight of stem found greater for cow dung extract in CD_3 (11.84 g/plant) and the lowest found in CD_1 (8.16 g/plant).For nutrient solution higher fresh weight of stem was in S_4 (11.98 g/plant) and lowest in S_1 (7.45 g/plant). It was revealed that balanced nutrition and optimum level of nitrogen ensured maximum vegetative growth resulting highest fresh weight/ plant. The results obtained earlier by Tittonell *et al.* (2003), were similar with the present study. In case of root, higher fresh weight (33.18 g/plant) found in CD_4 which is statistically similar that of CD_3 and minimum fresh weight (23.41 g/plant) was in CD_1 for cow dung extract. In response to nutrient solution higher root fresh weight found in S_4 (29.92 g/plant) and lowest in S_1 (26.47 g/plant).

In case of combined effect of cow dung extract and nutrient solution, the significant variation of fresh weight was found at harvesting (Table 5). The highest fresh weight in all cases were found in CD_3S_3 (35.13 g/plant) which were statistically similar that of CD_4S_4 (33.83 g/plant) and lowest were found in CD_1S_1 (21.20 g/plant).

Table 4. Main effects of cow dung extract and nutrient solution on fresh weight of lettuce

Treatments	Fresh weight (FW) per pant at harvesting time (g)			
	Total	Leaf	Stem	Root
Cow dung extract (CDS)				
CD_1	85.19 d[z]	53.63 d	8.16 d	23.41 d
CD_2	95.26 c	58.66 c	9.12 c	27.46 b
CD_3	112.84 a	71.02 a	11.84 a	29.98 a
CD_4	110.0 b	66.08 b	10.74 b	33.18 a
Nutrient solution (S)				
S_1	80.91 d	46.99 d	7.45 d	26.47 d
S_2	96.95 c	59.74 c	8.93 c	28.28 c
S_3	111.00 b	70.16 b	11 48 b	29.36 b
S_4	117.63 a	75.73 a	11.98 a	29.92 a
Level of significance (*P*)				
CD	<0.001	0.002	<0.001	<0.001
S	<0.001	<0.001	<0.001	0.146

[z]Means with different letter is significantly different by Tukey's test at $P \leq 0.05$. CD_1 = 50 g dry cow dung equivalent extract per liter, CD_2= 100 g dry cow dung equivalent extract per liter, CD_3 = 150 g dry cow dung equivalent extract per liter, and CD_4 = 200 g dry cow dung equivalent extract per liter.S_1 = 30% of the standard solution, S_2 = 40% of the standard solution, S_3 = 50% of the standard solution and S_4 = 60% of the standard solution. *P* represents the level of significance of ANOVA

Table 5. Combined effects of cow dung extract and nutrient solution on fresh weight of lettuce

Treatments	Fresh weight (FW) per pant at harvesting time (g)			
	Total	Leaf	Stem	Root
CD_1S_1	63.26 j[z]	36.91 j	5.14 l	21.20 j
CD_1S_2	78.75 i	49.63 h	6.83 k	22.28 ij
CD_1S_3	91.44 gh	57.57 f	9.75 f	24.12 hi
CD_1S_4	1.0731E e	70.39 d	10.89 e	26.03 fgh
CD_2S_1	75.50 i	42.53 i	7.16 k	25.80 gh
CD_2S_2	89.33 h	54.30 fg	7.97 j	27.05 fg
CD_2S_3	100.83 f	62.88 e	10.02 f	27.91 ef
CD_2S_4	115.37 c	74.93 c	11.34 d	29.10 de
CD_3S_1	93.96 g	53.90 g	9.07 g	30.98 cd
CD_3S_2	111.57 cd	67.89 d	10.89 de	32.78 bc
CD_3S_3	130.59 a	81.91 a	13.55 a	35.13 a
CD_3S_4	128.05 a	80.39 ab	13.83 a	33.83 ab
CD_4S_1	90.94 gh	54.62 fg	8.42 h	27.90 ef
CD_4S_2	108.15 de	67.11 d	10.03 f	31.01 cd
CD_4S_3	119.82 b	78.27 bc	12.59 b	30.30 d
CD_4S_4	119.82 b	77.20 bc	11.90 c	30.72 d
P	<0.001	<0.001	<0.001	<0.001

[z]Means with different letter is significantly different by Tukey's test at $P \leq 0.05$. CD_1 = 50 g dry cow dung equivalent extract per liter, CD_2= 100 g dry cow dung equivalent extract per liter, CD_3 = 150 g dry cow dung equivalent extract per liter, and CD_4 = 200 g dry cow dung equivalent extract per liter.S_1 = 30% of the standard solution, S_2 = 40% of the standard solution, S_3 = 50% of the standard solution and S_4 = 60% of the standard solution. *P* represents the level of significance of ANOVA

4.1.5 Dry weight of lettuce

Plant dry weights of lettuce were not varied significantly at transplanting time but difference in harvesting across treatments of cow dung (Table 6). Average total dry weight was higher in CD_4 (6.68 g/plant) and the lowest dry weight found in CD_1 (4.90 g/plant). In case of dry leaf higher weight found in CD_3 (3.95 g/plant) and the lowest leaf dry weight found in CD_1 (2.99 g/plant). Dry weight of stem found greater (0.74 g/plant) in CD_3 and the lowest dry weight of stem (0.51 g/plant) found in CD_1., On the other hand, higher root dry weight (1.98 g/plant) found in CD_4 and minimum dry weight (1.40 g/plant) found in CD_1.
Lettuce's dry weight differed significantly from four treatments of nutrient solution (Table 6) as well. Upon drying the harvested lettuce plant, the highest total dry weight was in S_4 (6.73 g/plant) and lowest was in S_1 (4.64 g/plant). In case of dry weight of leaf found higher (4.20 g/plant) in S_4 and the lowest leaf dry weight (2.61 g/plant) found in S_1. Dry weight of stem found greater (0.75 g/plant) in S_4 and the lowest dry weight of stem (0.47 g/plant) found in S_1. In case of root, higher dry weight (1.77 g/plant) found in S_4 and minimum dry weight (1.64 g/plant) found in S_1. This might be because of the proportion of nutrient supply in the plants. Andriolo *et al.* (2005) stated that lettuce growth was affected by different strength of nutrient solution. The results of this experiment also compatible with that.

In the event of combined effect cow dung extract and nutrient solution (Table 7) the lowest plant dry weight for all cases were found in C_1S_1 (3.62 g/plant) and the highest were found in C_3S_3 (7.51 g/plant) witch was statistically similar that of C_3S_4 for dry weight of leaf, stem and total. The maximum vegetative growth had been helped to ensure the highest dry weight / plant and that can be obtained because of the interaction impact of various levels of inorganic and organic nutrition.

Table 6. Main effects of cow dung extract and nutrient solution on dry weight of lettuce

Treatments	Dry weight (DW) per pant at harvesting time (g)			
	Total	Leaf	Stem	Root
Cow dung extract (CDS)				
CD_1	4.90 d[z]	2.99 d	0.51 d	1.40 d
CD_2	5.43 c	3.21 c	0.58 c	1.64 c
CD_3	6.44 b	3.95 a	0.74 a	1.75 b
CD_4	6.48 a	3.82 b	0.68 b	1.98 a
Nutrient solution (S)				
S_1	4.64 d	2.61 d	0.47 d	1.64 d
S_2	5.53 c	3.28 c	0.56 c	1.69 c
S_3	6.36 b	3.90 b	0.72 b	1.74 b
S_4	6.72 a	4.20 a	0.75 a	1.77 a
Level of significance (*P*)				
CD	<0.001	0.002	<0.001	<0.001
S	<0.001	<0.001	<0.001	0.148

[z]Means with different letter is significantly different by Tukey's test at $P \leq 0.05$. CD_1 = 50 g dry cow dung equivalent extract per liter, CD_2= 100 g dry cow dung equivalent extract per liter, CD_3 = 150 g dry cow dung equivalent extract per liter, and CD_4 = 200 g dry cow dung equivalent extract per liter.S_1 = 30% of the standard solution, S_2 = 40% of the standard solution, S_3 = 50% of the standard solution and S_4 = 60% of the standard solution. *P* represents the level of significance of ANOVA

Table 7. Combined effects of cow dung extract and nutrient solution on dry weight of lettuce

Treatments	Dry weight (DW) per pant at harvesting time (g)			
	Total	Leaf	Stem	Root
C_1S_1	3.62 m[z]	2.03 k	0.32 m	1.27 l
C_1S_2	4.52 k	2.76 i	0.43 l	1.33 k
C_1S_3	5.27 ih	3.22 g	0.61 g	1.44 j
C_1S_4	6.21 e	3.95 c	0.69 e	1.56 i
C_2S_1	4.3l	2.32 j	0.45 k	1.54 i
C_2S_2	5.15 j	3.02 h	0.51 j	1.62 h
C_2S_3	5.94 g	3.46 f	0.63 f	1.67 g
C_2S_4	6.50 d	4.04 c	0.72 d	1.74 f
C_3S_1	5.35 h	3.01 h	0.55 h	1.79 e
C_3S_2	6.43 d	3.78 d	0.68 e	1.97 c
C_3S_3	7.51 a	4.55 a	0.86 a	2.10 a
C_3S_4	7.43 a	4.49 a	0.87 a	2.07 b
C_4S_1	5.26 i	3.05 h	0.53 i	1.68 g
C_4S_2	6.06 f	3.57 e	0.63 f	1.86 d
C_4S_3	6.90 b	4.36 b	0.79 b	1.75nf
C_4S_4	6.76 c	4.28 b	0.75 c	1.73 f
P	<0.001	<0.001	<0.001	<0.001

[z]Means with different letter is significantly different by Tukey's test at $P \leq 0.05$. CD_1 = 50 g dry cow dung equivalent extract per liter, CD_2= 100 g dry cow dung equivalent extract per liter, CD_3 = 150 g dry cow dung equivalent extract per liter, and CD_4 = 200 g dry cow dung equivalent extract per liter.S_1 = 30% of the standard solution, S_2 = 40% of the standard solution, S_3 = 50% of the standard solution and S_4 = 60% of the standard solution. *P* represents the level of significance of ANOVA

4.3 Physiological growth traits

Significant variation of physiological growth parameters of lettuce plants was recorded with application of different levels of cow dung extract and nutrient solution (Table 8). In case of leaf area (LA), the higher (321.33 cm^2) leaf area was found in the plants grown in CD_4 and the lower (250 cm^2) was found in CD_1. Leaf area is an important determinant of light interception and consequently of transpiration, photosynthesis and plant productivity (Dufour, L. and Guérin, V. (2005). In case of Leaf Mass Ratio (LMR), the higher (0.921 g g^{-1}) Leaf Mass Ratio (LMR) was found in CD_3 and the lower (0.907 g g^{-1}) was found in CD_2. Higher LMR is one of the important criteria for producing higher metabolites. Prieto *et al.* (2007) reported that increased LMR gave the plants an increased ability to intercept light. In case of Leaf Area Ratio (LAR), the lower (106.79 cm^2 g^{-1}) Leaf Area Ratio (LAR) was found in CD_4 while the highest (161.20 cm^2 g^{-1}) was found in CD_2. Lower LAR is one of the important criteria for producing higher metabolites. In case of Root Weight Ratio (RWR), the lower RWR (0.0793250 g g^{-1}) was found in CD_3 while the higher (0.092878 g g^{-1}) was found in CD_2. Lower RWR is one of the important criteria for producing higher metabolites. Net assimilation rate (NAR) and relative growth rate of lettuce of lettuce was also significantly affected by cow dung extract (Table 8). The highest net assimilation of lettuce was found in CD_4 (0.0000080 $gcm^{-2}d^{-1}$). On the other hand, CD_1 (0.0000028 $gcm^{-2}d^{1}$) showed the lowest net assimilation rate. It might be due to this experiment's environmental conditions, especially high luminosity and temperature, Prieto *et al.* (2007) reported that increased NAR gave the plants an increased ability to intercept light.

The highest relative growth rate (RGR) of lettuce was found in CD_4 (0.00085 g $g^{-1}d^{-1}$). On the other hand, CD_1 (0.00045 g $g^{-1}d^{-1}$) showed the lowest relative growth rate. The results revealed that the highest relative growth rate was found in S_2. Meanwhile S_1 denoted the lowest relative growth rate.

In case of nutrient solution the highest leaf area (LA), root weight ratio (RWR), net assimilation rate (NAR) and relative growth rate (RGR) of lettuce was found in S_4 while leaf area ratio (LAR) height in S_3 and higher leaf mass ratio (LMR) found in S_1. The lowest leaf area (LA), leaf area ratio (LAR), root weight ratio (RWR), net assimilation rate (NAR) and relative growth rate (RGR) of lettuce were found in S_1, with the exception of the lowest leaf mass ratio (LMR) in S_4.

The physiological growth parameters of lettuce had been substantially affected by the combination of cow dung extract and nutrient solution treatment (Table 9). For the leaf area (LA), highest leaf area (LA) was found in CD_4S_4 (340.00 cm^2) and the lowest was found in CD_1S_1 (231.0 cm^2). The highest leaf area ratio (LAR) of lettuce was found in CD_2S_4 (167.202 $cm^2\ g^{-1}$) and the lowest found in CD_4S_2 (104.745 $cm^2\ g^{-1}$). In case of leaf mass ratio (LMR), the lowest leaf mass ratio (LMR) was found in in CD_2S_4 (0.905 gg-1) while the higher was found in CD_1S_1 (0.925 gg-1). The lowest root weight ratio of lettuce was found in CD_3S_1 (0.075247 gg_{-1}). On the other hand, the highest root weight ratio was found in CD_2S_4 (0.094552 gg_{-1}). Lettuce's net assimilation rate (NAR) and relative growth rate (RGR) are the lowest in CD_1S_1 and are 0.00000281 $gcm^{-2}d^{-1}$ and 0.000426 $g\ g^{-1}d^{-1}$ respectively. On the other hand, the maximum net assimilation rate was demonstrated in CD_4S_3 (0.00000815 $gcm^{-2}d^{-1}$) and relative growth rate in CD_4S_4 (0.000880 $g\ g^{-1}d^{-1}$).

Table 8. Main effects of cow dung extract and nutrient solution on physiological traits of lettuce

Treatments	LA (cm^2)	LMR ($g\ g^{-1}$)	LAR ($cm^2\ g^{-1}$)	RWR ($g\ g^{-1}$)	NAR ($g\ cm^{-2}\ d^{-1}$)	RGR ($g\ g^{-1}\ d^{-1}$)
Cow dung extract						
CD_1	250.00 d[z]	0.916 ab	157.404 b	0.08534 bc	0.0000028 c	0.00045 d
CD_2	270.00 c	0.907 c	161.20 a	0.092878 a	0.0000029 c	0.00047 c
CD_3	310.33 b	0.921 a	120.9 c	0.079325 c	0.0000061 b	0.00073 b
CD_4	321.33 a	0.913 bc	106.79 d	0.087490 ab	0.0000080 a	0.00085 a
Solution (S)						
S_1	268.25 d	0.917 a	133.51 b	0.08382 a	0.00000478 b	0.00060 c
S_2	278.67 c	0.914 ab	133.83 b	0.08530 a	0.00000499 ab	0.00062 d
S_3	297.25 b	0.913 ab	138.32 a	0.08650 a	0.00000506 a	0.00064 a
S_4	307.5 a	0.910 b	140.31 a	0.08941 a	0.00000507 a	0.00066 a
Level of significance (p)						
CD	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
S	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[z]Means with different letter is significantly different by Tukey's test at $P \leq 0.05$. CD_1 = 50 g dry cow dung equivalent extract per liter, CD_2= 100 g dry cow dung equivalent extract per liter, CD_3 = 150 g dry cow dung equivalent extract per liter, and CD_4 = 200 g dry cow dung equivalent extract per liter.S_1 = 30% of the standard solution, S_2 = 40% of the standard solution, S_3 = 50% of the standard solution and S_4 = 60% of the standard solution. *P* represents the level of significance of ANOVA.

Table 9. Combined effects of cow dung extract and nutrient solution on physiological traits of lettuce

Treatments	LA (cm^2)	LAR ($cm^2\ g^{-1}$)	LMR ($g\ g^{-1}$)	RWR ($g\ g^{-1}$)	NAR ($g\ cm^{-2}\ d^{-1}$)	RGR ($g\ g^{-1}\ d^{-1}$)
CD_1S_1	231.0 i	152.800 c	0.925 a	0.079207 ab	0.00000281 d	0.000426 h
CD_1S_2	239.0 hi	152.241 c	0.917 abc	0.082885 ab	0.00000 292 d	0.000446 gh
CD_1S_3	261.0 fg	161.114 abc	0.913 abc	0.08656 ab	0.00000 285 d	0.000460 fgh
CD_1S_4	269.0 ef	163.461 ab	0.907 bc	0.092732 a	0.00000 286 d	0.000466 fg
CD_2S_1	250.0 hg	154.255 bc	0.908 abc	0.091955 ab	0.00000 298 d	0.000460 fgh
CD_2S_2	262.0 f	158.679 abc	0.906 abc	0.091433 ab	0.00000295 d	0.000470 fg
CD_2S_3	278.0 e	164.688 a	0.906 c	0.093572 a	0.00000291 d	0.000479 fg
CD_2S_4	278.0 d	167.202 a	0.905 c	0.094552 a	0.00000294 d	0.000493 f
CD_3S_1	290.0 d	121.936 d	0.92 ab	0.075247 b	0.00000555 c	0.000676 e
CD_3S_2	301.33 cd	119.664 d	0.921 abc	0.078997 ab	0.00000559 bc	0.000716 d
CD_3S_3	319.0 b	119.942 d	0.921 abc	0.078917 ab	0.00000631 b	0.000756 c
CD_3S_4	319.0 a	120.826 d	0.916 abc	0.084138 ab	0.00000646 b	0.000780c
CD_4S_1	301.33 c	105.053 e	0.911 abc	0.088908 ab	0.00000779 a	0.000816 b
CD_4S_2	312.33 bc	104.745 e	0.912 abc	0.087865 ab	0.00000810 a	0.000850 ab
CD_4S_3	331.0 a	107.566 e	0.913 abc	0.086971 ab	0.00000815 a	0.000880 a
CD_4S_4	340.0 a	109.784 e	0.914 abc	0.086216 ab	0.00000803 a	0.000880 a
P	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

[z]Means with different letter is significantly different by Tukey's test at $P \leq 0.05$. CD_1 = 50 g dry cow dung equivalent extract per liter, CD_2= 100 g dry cow dung equivalent extract per liter, CD_3 = 150 g dry cow dung equivalent extract per liter, and CD_4 = 200 g dry cow dung equivalent extract per liter.S_1 = 30% of the standard solution, S_2 = 40% of the standard solution, S_3 = 50% of the standard solution and S_4 = 60% of the standard solution. *P* represents the level of significance of ANOVA.

4.4 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 (Table 10). 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 due to the amount and composition of the nitrogen. 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 witch 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) and Petropoulos *et al.,* (2008), this experimental findings 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 (Table 11). 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. In C_4S_4 (265.24 mg kg^{-1} FW) maximum nitrate content was determined which is statistically and similar with the treatments C_4S_3 (263.48 mg kg^{-1} FW) and C_3S_4 (262.24 mg kg^{-1} FW). The lowest nitrate content was determined in C_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. Similar findings were also reported earlier by Pôrto *et al*. (2008)

Table 10. Main effects of cow dung extract and nutrient solution on SPAD value and nitrate content in lettuce.

Treatment	SPAD index	Nitrate content (mg kg^{-1} FW)
Cow dung extract (CD)		
CD_1	13.57 c[z]	245.95 b[z]
CD_2	14.08 b	251.46 ab
CD_3	14.56 a	255.48 a
CD_4	14.80 a	254.24 a
Nutrient solution (S)		
S_1	12.94 c	265.95 b
S_2	13.67 b	277.46 ab
S_3	15.02 a	280.48 a
S_4	1.540 a	281.24 a
Level of significance (p)		
CD	<0.001	<0.001
S	<0.001	<0.001

[z]Means with different letter is significantly different by Tukey's test at $P \leq 0.05$. CD_1 = 50 g dry cow dung equivalent extract per liter, CD_2= 100 g dry cow dung equivalent extract per liter, CD_3 = 150 g dry cow dung equivalent extract per liter, and CD_4 = 200 g dry cow dung equivalent extract per liter.S_1 = 30% of the standard solution, S_2 = 40% of the standard solution, S_3 = 50% of the standard solution and S_4 = 60% of the standard solution. *P* represents the level of significance of ANOVA.

Table 11. Combined effects of cow dung extract and nutrient solution on SPAD value and nitrate content in lettuce

Treatment	SPAD index	Nitrate content (mg kg^{-1} FW)
C_1S_1	12.63 g[z]	245.95 f
C_1S_2	13.08 efg	248.48 def
C_1S_3	14.19 cde	250.48 cdef
C_1S_4	14.39 cde	261.24 abc
C_2S_1	12.71 fg	247.62 ef
C_2S_2	13.62 defg	252.46 bcdef
C_2S_3	14.80 bcd	256.61 abcdef
C_2S_4	15.20 abc	257.24 abcdef
C_3S_1	13.26 efg	251.95 bcdef
C_3S_2	14.00 cdef	253.46 bcdef
C_3S_3	15.22 abc	257.81 abcde
C_3S_4	15.77 ab	262.24 ab
C_4S_1	13.13 efg	255.95 abcdef
C_4S_2	13.97 cdef	259.46 abcd
C_4S_3	15.84 ab	263.48 ab
C_4S_4	16.26 a	265.24 a
P	<0.001	<0.001

[z]Means with different letter is significantly different by Tukey's test at $P \leq 0.05$. CD_1 = 50 g dry cow dung equivalent extract per liter, CD_2= 100 g dry cow dung equivalent extract per liter, CD_3 = 150 g dry cow dung equivalent extract per liter, and CD_4 = 200 g dry cow dung equivalent extract per liter.S_1 = 30% of the standard solution, S_2 = 40% of the standard solution, S_3 = 50% of the standard solution and S_4 = 60% of the standard solution. *P* represents the level of significance of ANOVA

4.5 Ascorbic acid content

Ascorbic acid content of lettuce was significantly affected by addition of different levels of cow dung extract (Figure 7). In case of cow dung extract the highest ascorbic acid content of lettuce was found in CD_4 (45.41 cc). On the other hand the lowest ascorbic acid content of lettuce was found in CD_1 (21.00 cc). In this present experiment, it was observed that, ascorbic acid content increased with increasing levels of cow dung extract.

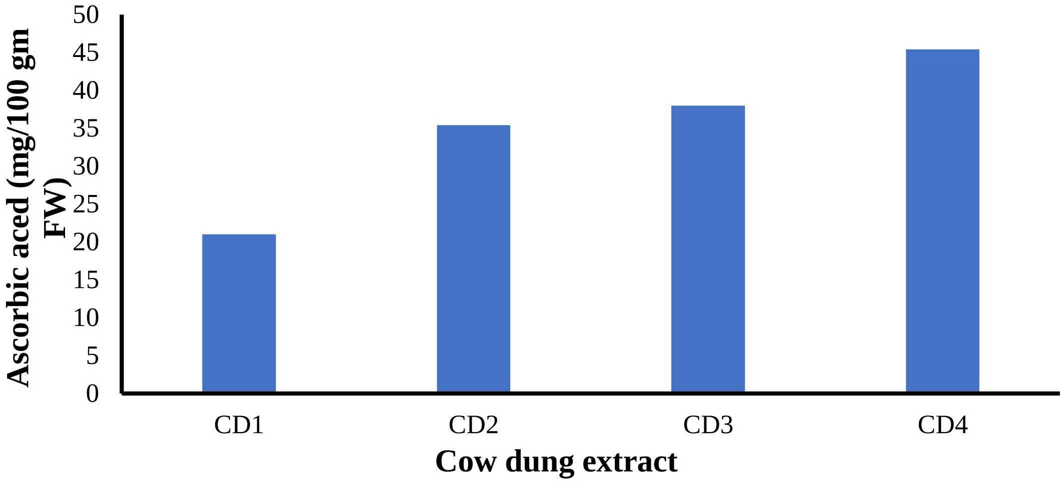

Figure 7. Effects of cow dung extract on ascorbic acid of lettuce

Here, CD_1 = 50 g dry cow dung equivalent extract per liter, CD_2= 100 g dry cow dung equivalent extract per liter, CD_3 = 150 g dry cow dung equivalent extract per liter, and CD_4 = 200 g dry cow dung equivalent extract per liter.

Ascorbic acid content of lettuce was significantly affected by addition of modified strength of nutrient solution (Figure 8). For nutrient solution, the highest ascorbic acid content of lettuce was found in S_4 (35.41 cc). On the other hand, the lowest ascorbic acid content of lettuce was found in S_1 (27.25 cc). Shinohara *et al.* (1981) reported that ascorbic acid content of lettuce was increased when grown in ¼strength 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 this results.

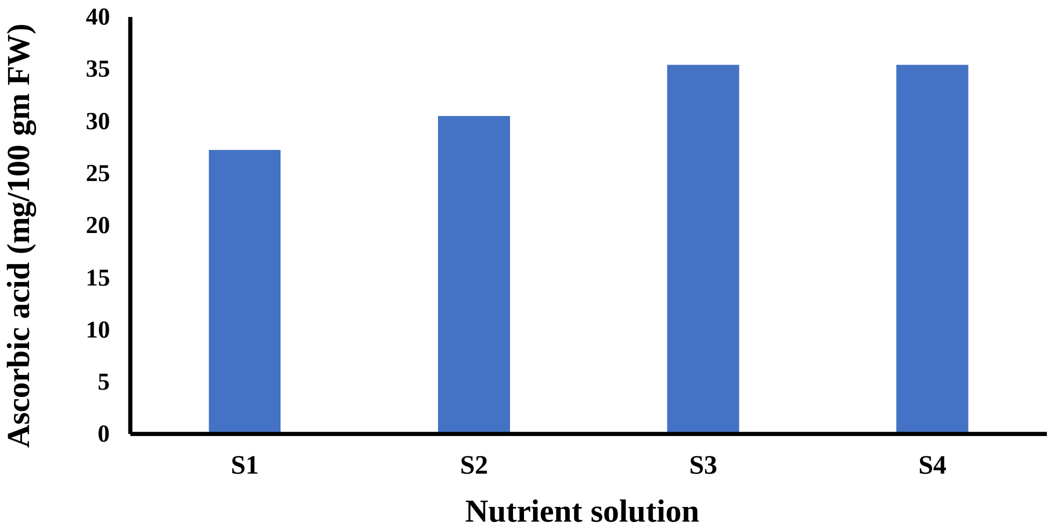

Figure 8. Effects of nutrient solution on ascorbic acid of lettuce

Here, S_1 = 30% of the standard solution, S_2 = 40% of the standard solution, S_3 = 50% of the standard solution and S_4 = 60% of the standard solution.

There was a significant interaction between cow dung extract and nutrient solution in case of ascorbic acid concentration on lettuce (Figure 9). The lowest ascorbic acid content of lettuce was found in C_1S_1 (18 cc). On the other hand, the highest ascorbic acid content of lettuce was found in C_4S_4 (50 cc) and the relatively similar ascorbic acid concentration in C_4S_3 (47 cc) and C_3S_4 (47 cc) 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.

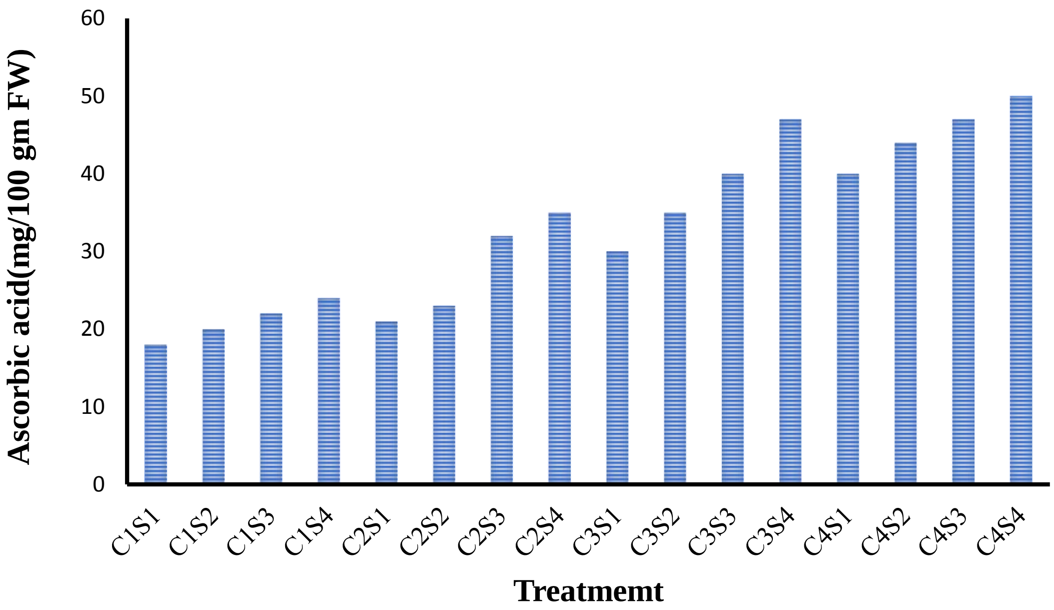

Figure 9. Combined effects of cow dung extract and nutrient solution on ascorbic acid of lettuce

Here, CD_1 = 50 g dry cow dung equivalent extract per liter, CD_2= 100 g dry cow dung equivalent extract per liter, CD_3 = 150 g dry cow dung equivalent extract per liter, and CD_4 = 200 g dry cow dung equivalent extract per liter. S_1 = 30% of the standard solution, S_2 = 40% of the standard solution, S_3 = 50% of the standard solution and S_4 = 60% of the standard solution.

4.6 β- Carotene content:

Plant carotenoids are the primary dietary source of provitamin A worldwide, with β- carotene as the most well-known provitamin A carotenoid. β- carotene content in lettuce plant increased with the increasing levels of cow dung extract (Figure 10). β- Carotene content was higher in the plants grown in CD_4 (239.00 μg\100g) on the other hand the lowest β- Carotene content of lettuce was estimate in CD_1 (152.53 μg\100g). Amin Ismail and Cheah Sook Fun (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.

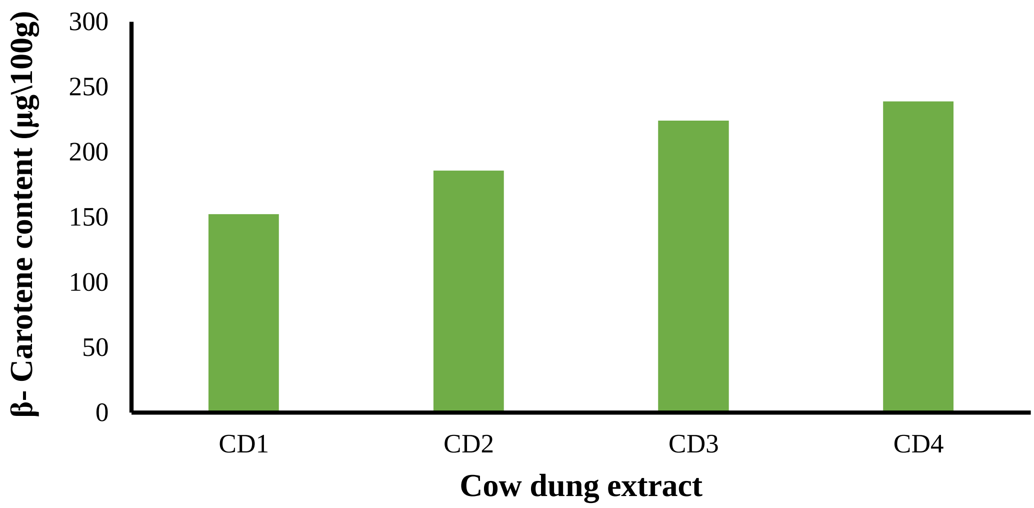

Figure 10. Effects of cow dung extract on β- Carotene content of lettuce

Here, CD_1 = 50 g dry cow dung equivalent extract per liter, CD_2= 100 g dry cow dung equivalent extract per liter, CD_3 = 150 g dry cow dung equivalent extract per liter, and CD_4 = 200 g dry cow dung equivalent extract per liter.

β- Carotene content of lettuce was significantly affected by addition of modified strength of nutrient solution (Figure 11). In the present experiment, β- carotene content increased with increased concentration of Rahman and Inden (2012) solution. For nutrient solution, the highest β- carotene content of lettuce was estimate in S_4 (226.17 µg\100g). On the other hand, the lowest ascorbic acid content of lettuce was found in S_1 (173.00 µg\100g).

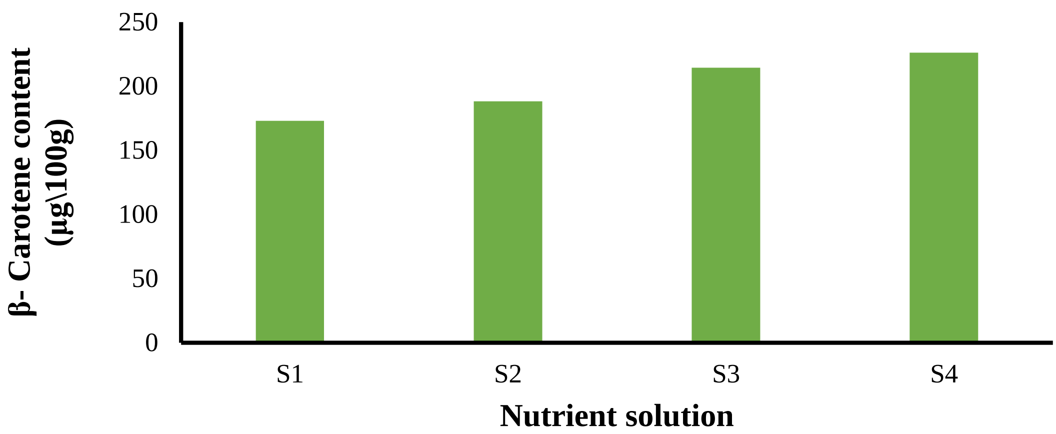

Figure 11. Effects of nutrient solution on β- Carotene content of lettuce

Here, S_1 = 30% of the standard solution, S_2 = 40% of the standard solution, S_3 = 50% of the standard solution and S_4 = 60% of the standard solution.

β- Carotene content of lettuce of lettuce had been substantially affected by the combination of cow dung extract and nutrient solution treatment (Figure 12). The lowest β- Carotene content observed in CD_1S_1 and the highest value was observed in CD_4S_4. In C_4S_4 (258.33 μg\100g) maximum β- Carotene content was determined which is relatively similar with the treatments C_4S_3 (253.67 μg\100g) and C_3S_4 (250.67 μg\100g) and they are statistically same. However, it was significant that β- Carotene content increased in the same treatment with higher yield.

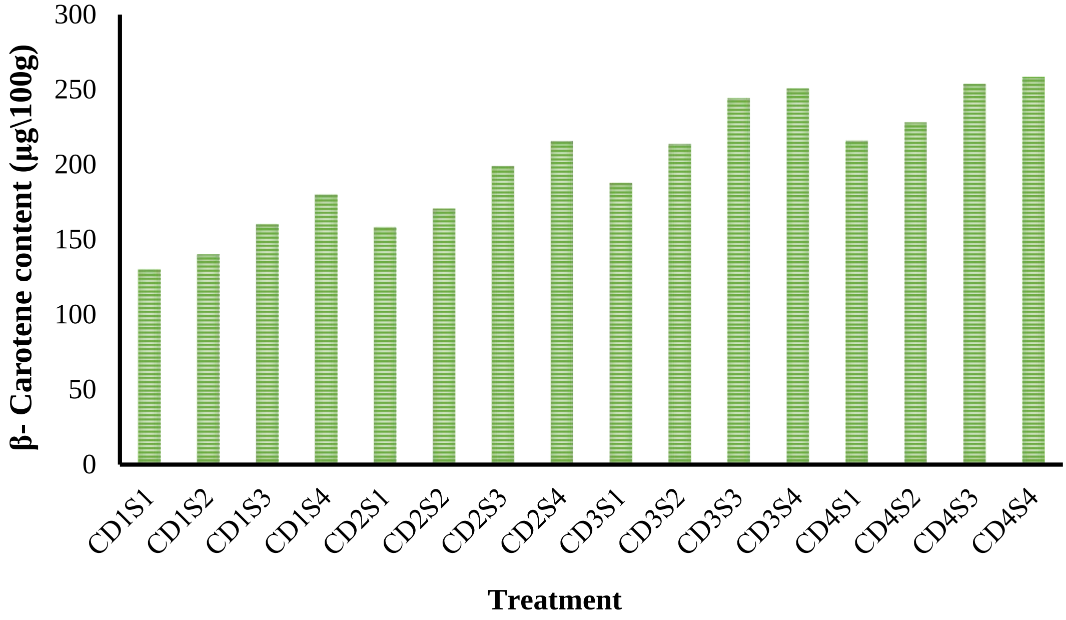

Figure 12. Combined effects of cow dung extract and nutrient solution on β-carotene content of lettuce

Here, CD_1 = 50 g dry cow dung equivalent extract per liter, CD_2= 100 g dry cow dung equivalent extract per liter, CD_3 = 150 g dry cow dung equivalent extract per liter, and CD_4 = 200 g dry cow dung equivalent extract per liter. S_1 = 30% of the standard solution, S_2 = 40% of the standard solution, S_3 = 50% of the standard solution and S_4 = 60% of the standard solution.

CHAPTER V

SUMMARY AND CONCLUSION

Hydroponic lettuce, like most leafy greens, can accumulate high levels of nitrates that are often harmful to human health**.** The accumulation of nitrate by plants is observed when plant nitrogen absorption exceeds the ability of assimilation and it is closely related to the amount and composition of the fertilizers applied. For that purpose, cow dung extract was used in addition to hydroponic nutrient solution. Since previous research indicates that treated cow dung extract is pathogen free and contains crop nutrients and it can used for the partial supplementation of NO_3 by NH_4. The aim of this study was to assess lettuce production and nitrate accumulation in it, using four types of aerated cow dung extracts (50 $g.L^{-1}$, 100 $g.L^{-1}$ 150 $g.L^{-1}$ and 200 $g.L^{-1}$) and four different strength (30%, 40%, 50% and 60%) of standard nutrient solution ratios. This study was conducted semi-green house at the Horticulture Farm of Sher-e - Bangla Agricultural University, Bangladesh during September 2018 to February 2019 for optimal and safe cultivation of hydroponic lettuce. Vegetative and physiological growth, yield, nitrate concentration on leaves were measured in the experiment. The summary was described here.

The results revealed that use of cow dung extract had significant effect on growth and quality yield of lettuce. In case of growth parameters of lettuce, such as plant height and leaf number, tallest plant (23.54 cm) and the maximum number leaves $plant^{-1}$ (16.66) were recorded from plant grown in CD_3 while the shortest plant height (18.57 cm)) and the minimum number leaves $plant^{-1}$ (14.34) were recorded from CD_1. But highest leaf breath (13.32 cm) was observed in CD_4 and lowest (11.18 cm) in CD_1. In case of fresh weight, maximum total fresh weight (116.0 g/plant) was recorded from the plant grown in CD_3 and minimum total fresh weight (85.19 g/plant) recorded from CD_1. In case of dry weight maximum total dry weight (6.68 g/plant) was recorded from the plant grown in CD_4 and minimum (4.90 g/plant) from the plant grown in CD_1.

The results showed that the solution of hydroponic nutrient solution had a big impact on the growth and quality of lettuce. The increase of plant height was more or less incremental up to harvest, highest plant height (23.13 cm) was accounted for S_4 and the lowest (17.85 cm) one for S_1. The maximum number of leaves $plant^{-1}$ (17.01), higher leaf breadth (14.17) was recorded from S_4 while the minimum leaves $plant^{-1}$ (13.41) and lower leaf breadth (9.72 cm) from S_1 at harvest.

Different physiological variables; viz. in case of leaf mass ratio (LMR), higher leaf mass ratio was recorded from the plant grown in CD_3 and lower leaf mass ratio was recorded from the plant grown in CD_2; in case of leaf area ratio (LAR) and root weight ratio (RWR), statistically higher result found in CD_2. In case of net assimilation ratio NAR, and relative growth rate (RGR), highest value found in CD_4. In CD_4, the maximum SPAD value (14.80) and highest nitrate content (265.24 mg kg^{-1} FW) were determined where the minimum SPAD value (13.57) and lowest nitrate content (245.95 mg kg^{-1} FW) were in CD_1.

In case of hydroponic nutrient solution, maximum total fresh weight (112.05 g/plant) was recorded from S_4 and minimum (80.91 g/plant) recorded from the plant grown in S_1. For dry weight, the maximum total dry weight (6.73 g / plant) of the plant grown in S_4 and the minimum total dry weight (4.64 g / plant) of the plant grown in S_1 were recorded. In case of SPAD value and nitrate content, the maximum SPAD value (15.40) and highest nitrate content (281.24 mg kg^{-1} FW) were found in S_4 where the minimum SPAD value (12.94) and lowest nitrate content (265.95 mg kg^{-1} FW) were in S_1.

Results showed that the addition of hydroponic nutrient solution along with cow dung extract had a substantial impact on vegetative growth, physiological growth as well as on lettuce nitrate content. In case of almost all growth parameters the best results were found for plants grown in C_3S_4 followed by C_4S_4 and C_3S_3 where the lowest were found in C_1S_1. The results also showed that the nitrate concentration in the edible parts of lettuce was higher in C_4S_4 followed by C_4S_3 and C_3S_4.

The present has therefore concluded that fresh marketable yield and ascorbic acid concentration and nitrate accumulation were affected by different levels cow dung extract and Rahman and Inden (2012) nutrient solution. The combination of CD_3 (150 g dry cow dung equivalent extract per liter) with S_4 (60% of the standard solution) would be most favorable for growth performance of leaf lettuce and contain average quantity of ascorbic acid along with far less accumulation of nitrate.

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APPENDICES

Appendix 1. Analysis of variances of total and leaf fresh weight at harvesting time of lettuce

Source of variation	Degrees of freedom (df)	Sum squares for fresh weight of lettuce at harvesting time		Means squares for fresh weight of lettuce at harvesting time		F -value	
		Total	Leaf	Total	Leaf	Total	Leaf
Cow dung extract (CD)	3	9540.8	5759.9	3180.2	1919.9	1949.4	1480.8
Solution(S)	3	7067.7	2528.1	2355.90	842.71	1444.13	649.96
CD x S	9	547.106	317.03	60.79	35.22	37.26	27.16
Error	32	52.204	41.49	1.63	1.29		

Appendix 2. Analysis of variances of NO_3 content in edible part of lettuce

Source of variation	**Degrees of freedom (df)**	**Sum squares for NO_3**	**Mean squares for NO_3**	**F - value**
Cow dung extract (CD)	3	822.73	274.24	17.20
Solution(S)	3	619.58	206.52	12.96
CD x S	9	104.58	11.62	0.729
Error	32	510.00	15.93	

Appendix 3. Analysis of variances of ascorbic acid content in edible part of lettuce

Source of variation	Degrees of freedom (df)	Sum squares for ascorbic acid content	Mean squares for ascorbic acid content	F - value
Cow dung extract (CD)	3	973.75	324.58	567.07
Solution(S)	3	4208.75	1402.91	131.20
CD x S	9	148.75	16.52	6.68
Error	32	79.16	2.47	

Appendix 4. Analysis of variances of β- carotene content of lettuce

Source of variation	Degrees of freedom (df)	Sum squares for β- carotene content	Mean squares for β- carotene content	F - value
Cow dung extract (CD)	3	21107.06	735.69	17.20
Solution(S)	3	54619.23	206.52	12.96
CD x S	9	940.76	11.62	0.729
Error	32	446.11	13.94	

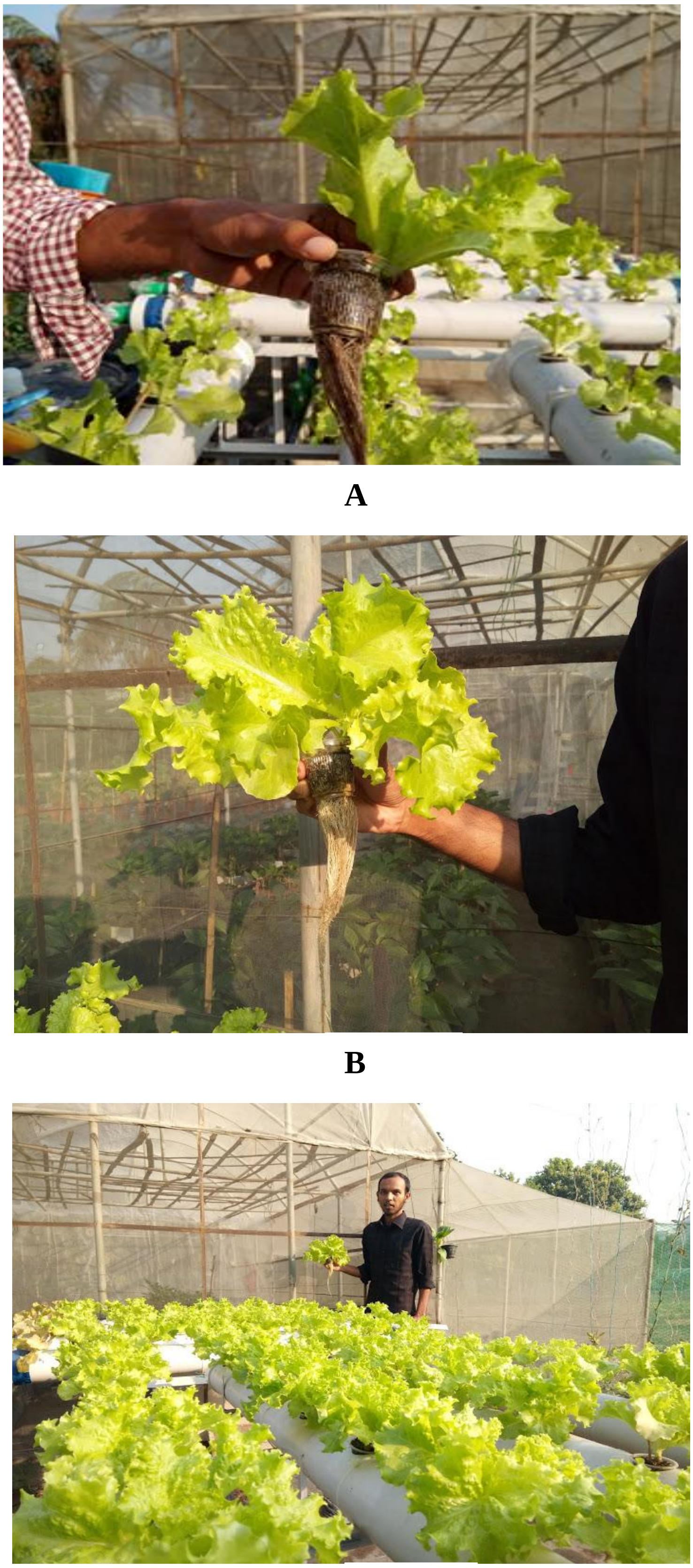
PLATES

A **B**

Plate 4. Seedling of lettuce in seedling trays (A: 4 days after sowing, B: Before transplanting)

A **B**

Plate 5. Application of nutrient solution (A: Cow dung extract, B: Solution)



A

B

C

Plate 6. Growing lettuce in hydroponic system (A: 14 days after transplanting; B: 21 days after transplanting; C: At harvesting stage)