EFFECTS OF ORGANIC AND INORGANIC FERTILIZERS ON YIELD AND ANTIOXIDANT PROPERTIES OF LETTUCEGROWN IN A ROOFTOP GARDEN

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CERTIFICATE

This is to certify that the thesis entitled“**EFFECTS OF ORGANIC AND INORGANIC FERTILIZERS ON YIELD AND ANTIOXIDANT PROPERTIES OF LETTUCE GROWN IN A ROOFTOP GARDEN”**Submitted to the Department of Agricultural Botany, Faculty of Agriculture, Sher-e-Bangla Agricultural University, Dhaka, in partial fulfillment of the requirements for the degree of **MASTER OF SCIENCE** in **AGRICULTURAL BOTANY**, embodies the result of a piece of bona fide research work carried out by **MONIKA KUNDU**, being Registration No. **19-10122** under my supervision and guidance. No part of the thesis has been submitted for any other degree or diploma.

I further certify that such help or source of information, as has been availed of during the course of this investigation has been duly acknowledged.

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Dedicated to

My

Beloved Family

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ABSTRACT

A pot experiment was conducted in the rooftop garden of Agricultural Botany Department of Sher-e-Bangla Agricultural University to study the effects of organic and inorganic fertilizers on yield and antioxidant properties of lettuce during November 2019 to May 2020. The twelve (12) treatments are T_0 = Soil 100%, T_1= Soil 100% + Inorganic fertilizer, T_2 = Soil 80% + Cowdung 20%, T_3 = Soil 90% + Vermicompost 10% , T_4 = Soil 95% + Biochar 5% , T_5 = Soil 80% + Cow dung 20% + Inorganic fertilizer, T_6 = Soil 90% + Vermicompost 10% + Inorganic fertilizer, T_7= Soil 95% + Biochar 5% + Inorganic fertilizer, T_8 = Soil 70% + Cow dung 20% + Vermicompost 10% + Inorganic fertilizer, T_9 = Soil 75% +Cow dung 20% + Biochar 5% + Inorganic fertilizer, T_{10} = Soil 85%+ Vermicompost 10% + Biochar 5% + Inorganic fertilizer, T_{11} = Soil 65% + Cowdung 20% + Vermicompost 10% + Biochar 5% + Inorganic fertilizer were taken in this experiment. The experiment was laid out in Completely Randomized Design (CRD) with four replications. The results of this study showed the significant effect on shoot growth of lettuce to different treatments. Both organic and inorganic fertilizers significantly increased the growth of lettuce. The growth parameter such as plant height, leaf number, shoot fresh and dry weight increased with sole or combined application of cowdung, vermicompost and biochar in soil than control (T_0). But the addition of inorganic fertilizer with organic fertilizer exhibited negative effects on shoot growth but soil along with inorganic fertilizers (T_1) increased shoot growth than control (T_0). The highest fresh weight as yield of lettuce was recorded from the treatment of T_3 (Soil 90% + Vermicompost 10%) while the lowest yield (82.23 g) was obtained from control (T_0). The pattern of root growth was inconstant with the shoot growth to different treatment and suggested that inorganic fertilizers dominating the root growth than shoot growth. The different antioxidant compounds of lettuce leaf such as vitamin C, phenolic and flavonoid content increased with sole application of organic fertilizers and combined application of inorganic and organic fertilizers in more than control (T_0). But inorganic fertilizers decreased the vitamin C, phenolic and flavonoid content. These antioxidant compounds content were constant with the DPPH activity with low IC_{50} value. The IC_{50} values obtained from the DPPH radical scavenging assay. The IC_{50} values of DPPH radical scavenging activity highly correlated with total phenolic content and total flavonoid content. Therefore, the results recorded that sole application of organic fertilizers improved the yield and quality of lettuce than sole application of inorganic fertilizers, and even combined application of organic and inorganic fertilizers in soil media grown under rooftop garden conditions.

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ABBREVIATIONS

AEZ	Agro-Ecological one
cm	Centimeter
CV %	Percent Coefficient of Variation
CRD	Completely Randomized Design
DPPH	2, 2-diphenyl-1-picrylhydrazyl
et al.,	And others
e.g.	exempli gratia (L), for example
etc.	Etcetera
FAO	Food and Agricultural Organization
g	Gram (s)
mg	milligram (s)
ml	Milliliter
L	Litre
SFW	Shoot Fresh Weight
SDW	Shoot Dry Weight
RFW	Root Fresh Weight
RDW	Root Dry Weight
RL	Root Length
M. S.	Master of Science
LSD	Least Significant Difference
SAU	Sher-e-Bangla Agricultural University
SRDI	Soil Resource Development Institute
DF	Degrees of freedom
MS	Mean square
^{o}C	Degree Celsius
GAE	Gallic acid Equivalent
%	Percentage
QE	Quercetine
µg	Microgram
TFC	Total Flavonoid Content
TPC	Total Phenolic Content

CHAPTER 1
INTRODUCTION

Urban agriculture is gaining attention as rooftop farming which provides a solution to food security and adaptation to climate change (Gupta and Mehtha, 2017 and Islam *et al.,* 2020). Rooftop gardens makes a bridge between city dwellers and the nature thus increase urban resilience with changing environment. Different types of urban agriculture- vertical farming, community gardening and rooftop gardening have been implementing throughout the world including Bangladesh.

It was reported that 87% residential rooftops of Dhaka city is occupied by roof gardens which are not sustainable due to various technical difficulties including hostile roof environment for growing crops, no linkage between research and extension services, deficiency of model garden, appropriate plant growing media (Uddin *et al*., 2016). In plant growing media, suitable composition of soil and organic and inorganic fertilizers are the major concern to mitigate the roof garden growing crop yield loss.

Rooftop gardening is a part of urban agriculture which contributing to meet the urban food demand and stimulate the economy of the country as a specialized agriculture. There are various types of fruits and vegetables were grown in the roof garden including salad vegetable lettuce. Lettuce (*Lactuca saliva* L.) belongs to the family Compositae (Asteraceae) with a cluster of leaves varying considerably in shape, character and colour in different varieties. It contains protein, carbohydrate and vitamin A, vitamin C, thiamine and riboflavin and minerals calcium, phosphorus, iron etc.

Inorganic fertilizers have performed a significant role in raising crop production since the “green revolution” (Liu and Yan, 2010) however they are not a permanent solution for maintenance of crop yields (Vanlauwe *et al.,* 2010). Long-term excessive use of inorganic fertilizers may accelerate soil acidification, affecting both the soil biota and biogeochemical processes, thus posture an environmental risk and decreasing crop production (Aciego Pietri and Brookes, 2008). Organic fertilizers such as cowdung, compost and biochar, could therefore be useful tools to sustainably maintain or raise soil organic matter, preserving and increasing soil fertility and crop yield.

Cowdung is a very important source of organic fertilizer which is eco-friendly. It maintains soil health and it can enhance crop productivity by increasing soil fertility. Using cowdung effectively can contribute to minimize environmental degradation and also reduce greenhouse gas concentration (Raj *et al.,* 2014). Cowdung increases the organic carbon content of degrading soil which may lead to the improving activity of beneficial soil microorganisms as well as the fertility condition of soil by increasing the availability of nutrients for the plants from soil. Cowdung significantly improved the growth and yield of plants (Gudugi, 2013; Mehedi *et al.,* 2012).

Vermicompost is important organic fertilizer and it is a product of interactions between earthworm and microorganisms by corrosion of organic waste and the process is called vermicomposting (Arancon *et al.,* 2005). Advantages of applying vermicompost comprise high levels of nutrients; low levels of contaminants (Atiyeh *et al*., 2000; Garg *et al*., 2006) and increased surface area for colonization by microorganisms (Arancon *et al.,* 2005) and also increases soil fertility without polluting the soil, as well as the quantity and quality of harvested products (Castillo *et al*., 2002). In addition, recently it was reported that vermicompost is widely used in organic agriculture which increased plant growth and production including lettuce through improving soil physiochemical and biological properties (Manyuchiet *et al*., 2013; Alperet *et al*., 2017 and Frasetya *et al*., 2019).

Biochar is a carbon-rich material obtained from thermo chemical conversion of biomass in an oxygen-limited environment. Biochar has been described as a possible tool for soil fertility improvement, potential toxic element adsorption, and climate change mitigation (Stewart *et al*., 2013). The profitable effects of biochar on plant productivity and soil microbial population are connected to the improvement of specific surface area, cation exchange capacity, bulk density, pH, water, and nutrients within the soil matrix (Thies and Rillig, 2009). Indeed, several studies have shown that biochar application to soil can (i) improve soil physical and chemical properties (Sohi *et al*., 2009 and Mukherjee and Lal, 2013) (ii) enhance plant nutrient availability and correlated growth and yield (Biederman and Stanley Harpole, 2013 and Jeffery *et al*., 2011). A group researcher showed that the combined application of compost and biochar had a positive synergistic effect on soil nutrient contents and water-holding capacity under field conditions (Liu *et al*., 2012). It increased the yield of various crops such as sweet potato, tomato, lettuce etc. (Dou *et al*., 2012; Upadhyay *et al*., 2014; Trupiano *et al*., 2017; Suthar *et al*., 2018).

It has been reported that lettuce is one of the much popular leafy vegetables for fresh consumption and considered as the best source of health-promoting mixture such as vitamins A, vitamin C, calcium, iron, antioxidants including flavonoids that is anti-carcinogenic (Nicolle *et al.*, 2004 and Mulabagal *et al*., 2010). An antioxidant is a compound with the capability to minimise the harmfulness of the free radicals (Lobo *et al*., 2010). There is a tendency that organically produced foods have increased vitamin C and dry matter as well as a higher content of compounds with antioxidant action (Reganold and Wachter, 2016; Baranski *et al*., 2014; Williams *et al*., 2016). Many phenolic compounds are antioxidants that may contribute to decreasing human diseases (e.g., cancer and heart diseases). The advantage of eating a diet rich in fruits and vegetables has been partially attributed to the improved consumption of phenolic compounds with antioxidant properties (Ames *et al*. 1993).

However, to my knowledge no study has explained to evaluate the influence of inorganic and organic fertilizers in soil media on growth, yield and antioxidant properties of lettuce as rabi season crops grown under rooftop garden conditions. Considering the above factors, the present experiment was undertaken to study the following objectives.

- To measure the effects of sole and combined application of organic and inorganic fertilizers on growth and yield of lettuce; and
- To find out the effects of sole and combined application of organic and inorganic fertilizers on antioxidant properties of lettuce grown under roof top garden conditions.

CHAPTER 2

REVIEW OF LITERATURE

Lettuce is one of the more important leafy vegetables in Bangladesh, as well as in the world. The researcher of different countries had received much attention in the lettuce including Bangladesh. The growth and yield of lettuce are influenced by organic fertilizer as many other vegetables such as root and tuber crops as well as spices.

2.1 Effect of organic and inorganic fertilizers of the growth and yield of lettuce and others

Abraham and Lal (2002) observed an experiment on yield attributes, oil and protein content of mustard seed (var. I'usa Bold) in a cropping system of soybean (rabi)/fodder cowpea. Treatments were 33% recommended dosage of NPK. 100% recommended dosage of NPK and combinations of 33% NPK with farm fertilizer of Ventiiconipost, farm compost + poultry manure, phosphate solubilising bacteria (PSB) + Rhizobium or Azospirillum and PSB + foliar application of 33% cowdung and indicated that seed yield and biological yield were highest in the 100% NPK treatment (3486.0 and 13270.0 kg/ha, respectively). Seed oil and protein content arrived the highest levels after 33% NPK/PSB + cowsdung (33.06 %) and 33% NPK/PSB + Rhizohiwn or Aospirillwn (5.147 %). respectively. Soil organic carbon was greatest in the 33% NPK/farm compost + Vermicompost treatment (0.714%) comparison to 0.565 % in the unfertilized soil.

Adriana Hernández *et al*. (2010) was conducted an experiment of the influenced on total growth and leaf nutritional content in lettuce (*Lactuca sativa* L.). Here analyzed three character of fertilizer treatment where two organic and one inorganic. A linear model was capacitated for statistical analysis applying a completely randomized experimental design. Ca, Mg and Mn in leaf showed maximum amount in organic fertilization treatments. The vermicompost treatment showed a maximum performance of Mg, Fe, Zn and Cu and minimum Na in lettuce leaf amount when compared to compost usage. This study was to appreciate the growth response on lettuce (*Lactuca sativa* L.) plants behaved with 25-wk vermicompost and compost as organic fertilizers, and then to comparison them to urea, the traditional chemical fertilizer. Results will inspire farmers and vendors usage of both compost and vermicompost as organic fertilizers, as well as the increase of the consumers' confidence level of organic products.

Asaduzzaman *et al*. (2010) directed an experement in the field of Sher-e-Bangla Agricultural University, Dhaka, Bangladesh during the stage from October 2009 to January 2010 to detect the combined effect of mulch materials and organic manure on the growth and yield of lettuce. Four different mulch materials viz. Mo = No mulching, M_1 = Dry water hyacinth, M_2 = Black polythene and M_3 = Dry rice straw and four levels of organic manure viz. OMo= no organic manure, OM_1 = Cowdung (20 t/ha), OM_2 = Poultry manure (10 t/ha) and OM_3 = Vermicompost (10 t/ha) were further used as experimental variables. The results discover that most of the growth parameters were influenced by the mulch materials and organic manure. All the parameters viz. number of leaves /plant, leaf length (cm), leaf breath (cm), dry matter accumulation (%), yield (g/ plant) and yield (t/ha) accomplished better in event of M_2OM_3 (Black polythene + vermicompost: 10 (t/ha). Though the maximum gross and net returns were obtained from the M_2OM_3 and it was apparently from the high results that the treatment combination of M_2OM_3 was more profitable compared with other treatments but from economic point of view (Benefit cost ratio) treatment M_1OM_2 (Dry water hyacinth + poultry manure : 3.37) was above economic than the M_2OM_3.

Asai *et al*. (2009) reported that biochar is a organic fertilizers which improved rice grain yields at sites with minimum P presence, which might be due to increase saturated hydraulic conductivity of the top soil, xylem sap flow of the plant and response to N and NP inorganic fertilizer treatments. Minimum soil N content by biochar application in N deficient soils could be involving the increase C/N ratio; therefore it might decrease crop productivity mortally (Lehmann *et al*., 2003). However, some biochars carried important number of micronutrients. For example, pecan-shelled biochar carried greatest number of copper (Cu), magnesium (Mg) and zinc (Zn) than the soil (Novak *et al*., 2009).

Botrini *et al*. (2004) was reported an experiment the organic nutrition of lettuce in organic farming and noticed that lettuce showed better growth rates and qualitative characteristics, and highest N and P uptake with organic fertilizers compare to the inorganic fertilizers treatment. In addition, maximum nutrients intake were also associated with leaf characteristics of lettuce.

Carter *et al*. (2013) was observed a pot experiment over a three crop (lettuce-cabbage-lettuce) cycle on the growth of transplanted lettuce (*Lactuca sativa*) and Chinese cabbage (*Brassica chinensis*). Biochar application amounts to potting medium of 25, 50 and 150 g kg^{-1} were applied with and without locally available fertilizers (a mixture of compost, liquid compost and lake sediment). The biochar treatments were establish to increase the final biomass, root biomass, plant height and number of leaves in all the cropping cycles in compare to no biochar treatments. The maximum biomass increase due to biochar additions (903%) was found in the soils without fertilization, rather than fertilized soils (483% with the equivalent biochar application as in the "without fertilization" case). Over the cropping cycles the impact was decreased; a 363% increase in biomass was viewed in the third lettuce cycle.

Crane-Droesch *et al*. (2013) was conducted positive crop yield feedback as a result of biochar use over much of Sub-Saharan Africa, Southeast Asia, portion of South America and southeastern North America. Here noticed increase in crop yields in these extremely weathered and nutrient-poor soils could be explained by biochar soil amendments increasing soil aggregation, improving nutrients retention, and enhancing soil water wearing ability. Despite biochar‟s agronomic benefits, negative effects under biochar amendment on plant productivity have also been indicated in peat soils whereas moderate to negative yield response could be noticed in higher of the leading countries in grain production.

Dalila Trupiano *et al.* (2017) conducted an experiment on lettuce with effect of biochar. Effects of sole application of biochar and combination with organic fertilizer, such as compost, are not fully meaningful. Here tested the effects of biochar corrective, compost addition, and their combination on lettuce plants grown in a soil poor in nutrients. An initial screening was also done to evaluate the effect of biochar and compost toxicity, using cress plants and earthworms. Results showed that compost amendment had clear and positive effects on plant growth and yield and on soil chemical characteristics. Here found the results thus demonstrate that in a soil poor in nutrients the biochar alone could be effectively used to enhance soil fertility and plant growth and biomass yield.

Das *et al.* (2002) was conducted the thesis of vermicompost and chemical fertilizer application on the growth and yield of green gram. The dry matter and pod yield of green gram were increased with the application of vermiconipost applied in mobilized form. The yield was higher with 100% enriched vermicompost comparison to sole organic manure. The

Greater dry matter content was pod yield, nutrient uptake (N, P and K), plant height. leaf area, root volume, number of nodules and fresh weight of nodules were noticed with treatments containing vermicomipost. Flowering was previously by 7 days in vermicompost-treated plants comparison with the control.

Devi and Singh (2012) reported an experiment with different levels of chemical fertilizers (NPK) and vermicompost. NPK and vermicompost significantly affected the yield attributing characters and growth and biomass production in cabbage (*Brassica oleraceae* var. capitata) cv. Pride of India. There were six treatments *viz*. T_1: control i.e. without any fertilizer; T_2: NPK @140:140:140 kg/ha; T_3: NPK@105:105:105 kg/ha + Vermicompost @ 1 ton/ha; T4: NPK@70:70:70 kg/ha + Vermicompost @ 2 tons/ha; T_5: NPK@35:35:35 kg/ha + Vermicompost @ 3 tons/ha; T_6: Vermicompost @ 4 tons/ha). The highest of 58.67% increase in yield over control was observed in a combined application of NPK and vermicompost in half of their recommended doses.

FAO (2013) reported that urban agriculture is the practice of cultivating, processing, and distributing food in or around a village, town, or city. It can also include animal husbandry, aquaculture, agroforestry, urban beekeeping and horticulture. These activities happen in peri-urban areas as well as urban areas.

Getnet and Raja (2013) observed an experiment to produce vermicompost from organic solid wastes by using red earth worm and to check growth developing and pest suppression properties on cabbage (*Brassica oleracea)*. Vermicompost was used at the rate of 25, 50, 100 and 200 gm/plant individually. Each application 10 plants were selected and vermicompost application was expensive on bimonthly basis. Totally 40 plants were used for control group in which were 10 plants elected randomly. Meaningful differences ($p<0.05$; LSD) were noticed in the growth and development and number of plant height, cabbage head, leaves of cabbage comparison to control. Vermicompost have important effect on cabbage growth improve and minimise the aphid infestation. Evermore using vermicompost to all variety of crops and receiving it as mineral fertilizer may create job opportunity to small scale farming society. Also, in this ever increasing value of chemical fertilizers, the use of vermicompost seems to be perfectly feasible in agro-management and should be including as one of the elements of poverty alleviation strategies.

Ghuge *et al.* (2007) directed a field experiment to study the provoke of inorganic and organic sources of nutrients on growth, yield and quality parameters on cabbage. Combined effects of organic and inorganic fertilizer are more effective than the sole application. Between the difference combinations studied, performance of 50% RDF + 50% vermicompost @ 2.5 t/ha was proper among the rest of the treatments followed by 50 percent RDF + 50% Terracare @ 1.25 t/ha and 50% RDF + 50 percent organic booster @ 1.0 litre/plant in 4 splits above the control in respect of plant spread, head circumference, average yield head/plant, yield of head per hectare, chlorophyll content and compactness of head.

Hernandez *et al*. (2010) has reported an experiment of greenhouse study to evaluate the effect on total growth and leaf nutritional content in lettuce (*Lactuca sativa* L.). There are three types of fertilization treatments were analyzed: two organic and one inorganic. Vermicompost both compost were produce from cattle manure. The results showed imparity in weight and leaf content for the N and K variables and the maximum mean values for these variables were in the urea treatment. Leaf content of Ca, Mg, and Mn showed highest values in organic fertilization treatments. The vermicompost application showed a maximum contribution of Mg, Fe, Zn and Cu and minimum Na found in lettuce leaf when comparison to compost usage.

Hussein Jawad Moharrm AL-Bayati, *et al.* (2019) has published a research paper on organic and chemical fertilizer where they cultivars two lettuce variety. This is a RCBD factorial experiment and 12 treatments (2 x 2 x 3) were used. There were no significant differences showed in the results between the two varieties in maximum vegetative growth and yield while there were a significant competition of the 40 cm distance on the 30 cm distance in head circumference, leaf area, stem weight and mean marketable head weight while there were a significant competition of the treatment of 30 cm planting distance in terms of marketable and total yield. Here noted that no significant differences observed between the organic fertilization treatments with the normal chemical fertilizer plants in terms of vegetative and yield in all vegetative traits indicated traits expect the mean stem weight.

Iftekhar and Qasim (2003) was conducted an experiment of soil amendments made by using farmyard manure as main sources and by making different combinations with sand, silt and saw dust. Potting media in different combinations were better than the sole factor of the soil itself because different combinations of potting media produced more growth and vigor of the plants and improved total available N and P among various growths responses and soil and plant NPK contents.

Jeffery *et al*. (2011) observed -28% to 39% changes in plant productivity (crop yield and above-ground biomass) following biochar amendment to soils which are partly explained by biochar liming effect and improve soil moisture retention, associated with improved nutrient availability to plants. For biochar source"s effects on yield response, poultry litter indicated the strongest (significant) positive effect (28%), in contrast to biosolids, which were the only feedstock indication a statistically significant negative effect (-28%). Positive crop productivity happened in acidic than in neutral soils, in sandy than in loam and silt soils.

Johannessen *et al.* (2004) conducted an experiment that no difference in bacteriological quality could be discovered in lettuce at harvest after application of various types of manure-based fertilizers grown under Norwegian conditions. Significance and Impact of the Study, the results showed that the use of manure does not have important impact on the bacteriological nature of organic lettuce. However, others have suggested that there is highly risk by using the manure. There is a need another more research in the field.

Kamron (2006) has published a paper titled ‘Adoption of roof gardening at Mirpur-10 area under Dhaka city’. She published that the preferred distinctiveness of the respondents, family size, roof gardening knowledge, use of information sources, approach towards roof gardening and familiarity of roof gardening had encouraging result of relationship with their acceptance of roof gardening. Other characteristics namely: age, family education and family earnings did not show any important relationship with the respondent’s adoption of roof gardening.

Leon *et al.* (2012) was conducted an experiment of lettuce is the most important leaf vegetable grown in Argentina mainly in the green belts. This species demands 90 to 100 kg ha^{-1} nitrogen, which can be supplied by synthetic chemical fertilizers or organic supplements. They carried out a trail to evaluate the impacted of the application of vermicompost on the growth parameters of lettuce in two commercial types: leaf lettuce (cv Brisa) and butterhead (cv Daguan). During cultivation and at harvest measurements of fresh and dry weight, leaf number and area, nitrate and minimising sugar concentrations were made. At harvest, vermicompost addition influenced 6 nitrate content in leaf lettuce (cv Brisa) increasing its concentration. Yield was not influenced by vermicompost application.

Liu *et al.* (2013) was conducted an experiment of crops grown with biochar resulted with a 10.6% improve on average on dry land soils whereas a 5.6 % increase has been noticed for paddy rice. Effectiveness of biochar in increasing plant productivity is variable. Biochar application has been indicated to improve by about 10% plant productivity (Liu *et al*., 2013) and about 25% for aboveground biomass (Biederman and Harpole, 2013). Wang et al. (2014) also reported that biochar effectiveness on plant productivity differed considering variations in climate, soil properties, investigated crops, and experimental conditions.

M.B. Hossain and K.S. Ryu (2017) was conducted an experiment to identify the suitable dose of organic and inorganic fertilizer for lettuce production. Different doses of organic fertilizer (6.5, 13 and 26 t ha^{-1}) and the recommended dose of chemical fertilizer (RDCF) as standard were elected for this experiment. Organic matter extent was increased of 17.79, 43.82 and 89.89% in 6.5, 13 and 26 t ha^{-1} organic fertilizer treated plots gradually over recommended dose of chemical fertilizers. Organic fertilizer significantly increased leaf size (length and breadth) of lettuce than inorganic. Organic matter increased with the raised of pH in soil because pH are increases the negative charge on such surfaces is raised and repels negatively charged molecules into the soil solution, thereby increasing dissolve organic matter concentration. Positive and significant interrelation was observed on yield and yield quality of the lettuce and soil nitrogen, organic matter with pH, total nitrogen with mineral nitrogen and negative interrelation was found with applied organic fertilizer with cadmium and lead.

Masarirambi *et al.* (2010) was carried an experiment of organic fertilizers were (a) bounce back compost, (b) cattle manure and (c) chicken manure. The amount of application were 40 tons per hectare (t/ha) for chicken and cattle manures, 1.5 t/ha basal dressing and 1.0 t/ha side 7 dressing for bounce back compost. Inorganic fertilizers 2:3:2 (22) + 0.5 % zinc (Zn) and limestone ammonium nitrate (LAN 28%) were included at specific application amounts of 955 kg/ha basal dressing and 100 kg/ha side dressing as control. The results exposed that type of fertilizer used significantly ($P< 0.05$) affected growth, yield and nutritional quality of lettuce. A tendency in superiority of the various types of organic fertilizers was noticed as the chicken manure exhibited relatively higher values on number of leaves, plant height, marketable yield and mean leaf dry mass. Cattle manure was second, and after that bounce back compost and lastly the inorganic fertilizers. Results of this experiment discoved that inorganic fertilizers were less suitable in lettuce production in river sand when compared to organic fertilizers. It is recommended that lettuce can be grown effectively using organic fertilizers.

Morra *et al.* (2003) conducted a research on organic manure of lettuce. They observed that growth rates of lettuce plants in the organic plots were maximum than those of plants grown under inorganic (100-150 kg N/ha) fertilization. Yields of saleable heads were maximum in the two organic plots (51 and 60 t/ha) than inorganic fertilizer (36 t/ha) or control (30 t/ha) plots. Plants from the organic plots had the more nitrate contents on a dry matter basis, but to their highest water content, nitrate contents were equivalent to those of plants from inorganic manure and control plots on a fresh matter basis.

Prabhakaran and James-Pitchai (2002) was observed in the field and pot experiments to study possibility of substitution of N with organic N source *viz*., FYM, fresh mud, fish meal, pig manure, poultry manure to get superior quality fruits of tomato. Based on the N content of the organic N source on dry weight basis, the quantities necessary for the substitution of recommended dose of N at 50 and 100% level were worked out and applied. Application of organic N sources at both levels increased pH, TSS, titratable acidity, decreasing and non-deareasing sugar, crude protein and ascorbic acid content of tomato over no manure (control). Among the different organic N sources, application of recommended dose of N in the form of poultry manure recorded maximum pH, TSS, titratable acidity, reducing sugar, non-reducing sugar, crude protein and ascorbic acid amount in tomato fruits in both the experiments.

Rasoul *et al.* (2010) has published a report that vermicompost bear readily available nutrients such as nitrates, phosphates, and exchangeable calcium and soluble potassium. Vermicompost is an organic fertilizer where present more nutrient due to rich in humus, NPK, micronutrients; beneficial soil microbes- nitrogen fixing and phosphate are solubilized by bacteria‟ and growth hormones auxins, gibberellins and cytokinins. Here present more bacteria, actinomycet.es, fungi and cellulose- degrading bacteria. Vermicompost has showed the ability of influencing growth and productivity of plants.

Stintzing *et al.* (2002) conducted the field trial showed the pelleted broiler manure gave the best effect on yield than stored broiler manure. Nutrient balances reported that it was difficult to attain a good balance between application and uptake of nutrients when using broiler manure, especially pelleted. Soil samples inform that the amount of mineral nitrogen in the soil after harvest did not different significantly between the two broiler manures at the two levels of application.

The effect of three types of organic manures, its combination within and with chemical fertilizer was studied under the nylon net house. Cowdung (CD), chicken manure (CM) and duck manure (DM) were applied 4.5, 4.7 and 5.8 t/ha, respectively, contributing 81 kg nitrogen each. In the case of combination treatments each combination contributed 1:1 ratio of nitrogen. A treatment with recommended dose of chemical fertilizer (CF) providing 81 kg nitrogen was also included. The crop grown with 4.7 t/ha of CM alone and its combination with CF (2.35 ton CM plus 156 kg complete fertilizer i.e., 15-15-15 together with 82 kg/ha of ammonium sulphate) gave a significantly higher yield throughout the crop season. Combination treatments within organic manures did not establish any statistical significance. In spite of the differences in total yield, fibre content and dry matter percentages showed not significant results irrespective of treatments and crop seasons (Paudel *et al.*, 2004).

Tlustos *et al.* (2002) was conducted in a pot experiment to know the effect of slow release N fertilizers and urea with three vegetables (radish cv. Duo, lettuce cv. Detenicka and carrot cv. Nantes) and two rates of applied N. Right away applied fertilized radish, subsequently grown unfertilized lettuce and third crop straight fertilized carrot were treated by urea as control treatment and by three samples of slow release fertilizers based on urea formaldehyde condensate of different solubility. Availability of N from minimum release samples influenced yield of growing vegetables and their nitrogen uptake. Minimum availability of N caused lowest yields of radish and subsequently grown lettuce mainly on treatments with minimum rate of fertilizer comparison with urea treatments. Carrot planted as a third vegetable and straight treated by nitrogen showed highest yield at treatments with low soluble samples due to longer growing period and continuing release of N from slow soluble samples. Yield of dry matter of individual vegetables correlative to well with uptake of nitrogen determined by balance and isotope methods. Among the both isotope techniques introduced, about twice minimum utilization than the balance method probably because by priming effect of N and by unsuitable conditions for plant growth at unfertilized zero treatment.

Trupiano *et al*. (2017) tested the effects of biochar amendment, compost addition, and their combination on lettuce plants grown in a soil poor in nutrients. Compost amendment had been clean and positive impact on plant growth and yield and on soil chemical characteristics. However, biochar alone stimulated lettuce leaves number and total biomass, improving soil total nitrogen and phosphorus contents, as well as total carbon and improving related microbial communities. Combining biochar and compost, no positive synergic and summative effects were observed. It was recommended that in a soil poor in nutrients the biochar alone could be effectively used to enhance soil fertility and plant growth and biomass yield.

Lei *et al.* (2004) stated that the rules of nitrate accumulation in Dian Lake (Beijing, China) drainage area in intensive cultivation were studied. Results showed that fertilizer N was the prime cause of the accumulation of NO_3 in soil. The effects of P on NO_3 accumulation in soil differ from crops to crops. The fertilizer P input evidently influenced the accumulation of NO_3 in the soil of cultivating pimiento and the increase of fertilizer P input decreased NO_3 accumulation. The effects of P on NO_3 accumulation were different according to the changes of N input. No evident effects were observed on the NO_3 accumulation in the soil of cultivating lettuce with P input.

Jacnaksorn and Ikeda (2004) reported that in an attempt to reduce the hydroponic growing cost and to facilitate the preparation and source of nutrient solution, soil fertilizer was evaluated as a substitute for soilless nutrient solution in Osaka Prefecture, Japan in 1999. Comparisons of growth and nutrient uptake were made with pak choi (*Brassica chinensis*), lettuce (*Lactuca saliva*) and Chinese cabbage in deep flow technique (DFT) as and re-circulation nutrient film technique (NFT) treated with soilless nutrient solution (NSI) and soil fertilizer solution (NS2). The nutrient solution was chemically analyzed every week to monitor its change. Satisfactory results were achieved in all vegetables tested.

Feller *et al.* (2003) observed that bunching carrots, Japanese radish, dill, lambs' lettuce, rocket salad, celeriac and celery. Harvesting tabulates the average removal of nutrients by harvesting for N, P, K and Mg. Nitrogen demand and the N main target value in kg/ha arc compared with data published in 2001. Data are within a 10% variation range, however Japanese radish and celery had higher demands due to strong vegetative growth. The highest N demand was found in celery (270 kg N/ha), followed by Japanese radish (245 kg N/ha), spring onion (160 kg N/ha), bunching carrot (145 kg/ha), dill (110 kg N/ha), rocket salad (100 kg N/ha) and lambs' lettuce (38 kg N/ha). For rocket salad, nitrogen uptake curves modelled and measured arc presented for different sowing dates.

Nadasy (1999) was indicated that the greatest dry matter production found at 80mg/kg N. The fresh and dry weights were lesser after the application of calcium nitrate. Applying N in the ammonium form produced nearest results to applying both nitrate and ammonium forms. Dry matter production was best when both N forms were applied. Increasing N rates up to 320 mg/kg gradually upturned the N content of the lettuce leaves.

Singh *et al.* (2005) reported a study to assess the effect of vermicompost on cauliflower productivity and profitability considering soil health under small production systems. The farmers' reaction on the use of vermicompost was extremely positive because of its ingenuousness and compatibility with the farming system components and with the household inside resources, as well as its cost effectiveness. Moreover, vermicompost was also accepted by the resource-rich farmers who preferred to use vermicompost in place of chemical fertilizers due to environmental considerations and to campaign health hazards. The impact exploration revealed that approximately 55% of the cauliflower growers accepted vermicompost in adopted villages as well as in neighbouring areas.

2.2 Effect of organic and inorganic fertilizers on the antioxidant activities of lettuce

Bevly M. Mampholo *et al.* (2016) conducted an experiment on lettuce with photochemical and overall quality. Red lettuce varieties are rich in bioactive compounds and antioxidant property mainly due to higher total phenols and flavonoid compounds. Total phenolic content was generally higher in red lettuce varieties, Cantarix, Lunix and Multired 4 and ranged from 1.75 to 2.81 mg GAE/100 g, FW. On the hand the green lettuce varieties, among the green lettuce varieties; Multigreen 3 (Green Curly) and Multigreen 1 (Green Curly) showed higher total phenolic content (1.12 and 1.14 mg GAE/100 g, FW, respectively). The Green Oak varieties and Green Curly varieties, Multigreen 1 and Multigreen 3 showed higher concentration of flavonoid contents. DPPH and ABTS.1 assays were carried out to measure the free radical-scavenging activity of the lettuce extract. There were no significant variations observed between the red and green lettuce varieties with respect to antioxidant scavenging activity DPPH.

Chang Ha Park *et al.* (2018) conducted an experiment compared with red skirt and green cultivated lettuce. This results showed that the total phenolic content was higher in the red skirt methanol extracts with a level of 64.9 ± 0.3 mg GAE/g, and phenolic content of green skirt was 49.4 ± 0.3 mg GA/g. Here also found total flavonoid content in the methanol extract of red skirt showed the highest concentration (291.6 ± 9.0 mg rutin/g), followed by green skirt (223 ± 9.8 mg rutin/g). The red skirt showed a maximum content of anthocyanin (23.7 ± 0.8 mg/100 g) compared to the green skirt (7.4 ± 0.9 mg/100 g. Red skirt methanol extract proved 77.5 ± 0.4% 2,2-diphenyl-1-picrylhydrazyl (DPPH) activity, 50.8 ± 0.8% hydrogen peroxide radical scavenging assay and absorbance value (0.052) reducing ability assay at 250 mg/mL, having significantly highest activity than the green skirt extract. The red lettuce extract also showed highest phenolic compounds, flavonoids, and anthocyanins, and it possesses much antioxidant properties caparisoned to the green skirt extract.

Gan Y.Z. and Azrina A. (2016) was reported that the total phenolic content and total flavonoid content were determined as total antioxidant. The EC_{50} values received from the DPPH radical scavenging assay ranged from 303.56 to 4485.41 µg/ml. The red coral lettuce had the lowest EC_{50} value inform the highest antioxidant activity among the varieties. Total phenolic content of samples enlarge from 4.85 to 76.05 mg gallic acid equivalent/100 g fresh weight, with the red coral lettuce had the highest value. Total flavonoid quantity of the lettuce samples ranged from 2.28 to 21.96 mg quercetin equivalent/100 g fresh weight, and were significantly different ($p<0.05$) among the samples. The DPPH radical scavenging activity is greatly affected by the total phenolic content of the lettuce samples.

Gordana Acamovic-Djokovic *et al.* (2011) observed that lettuce is a highly rated vegetable in human nutrition not only for its richness in minerals and vitamins but also for the fact that nowadays it is produced all year round, and consumed fresh so that all the ingredients stay intact. Lettuce is not rich in vitamin C. As part of the study of antioxidant features of lettuce, vitamin C (L-ascorbic acid) was determined in several types of lettuce. Using the appropriate mixture of acids, total vitamin C was extracted from the samples, and the content of L-ascorbic acid was determined using the Tillman's method. Leaf lettuce Levistro (9.60mg/100g) and Kibou (5,25mg/100g) have a higher content of vitamin C than the head-forming type Butterhead (3.85mg/100g). The red-coloured oakleaf Murai contains the lowest amount of vitamin C (3.50mg/100g), whereas lettuce batavia-Temptation has a higher amount (4.99mg/100g). The lower vitamin C content in the red-leaf lettuce is in agreement with the data of other authors (Still 2007). The highest vitamin C content was determined in lettuce Levistro 9.60mg/100g of fresh lettuce, whereas Murai contained the lowest amount of vitamin C, 3.50mg/100g.

Herpandi *et al.* (2021) was conducted an experiment with lettuce on antioxidant activity. Here they were observed that free radicals including reactive oxygen species are continuously increasing in the human body. This study aimed to determine the antioxidant activity of fragment and analyzed the functional groups of water lettuce (*Pistia stratiotes*) methanol extract. The separation method was performed by using thin-layer chromatography (TLC) and column chromatography. The separated fractions were moderate for their antioxidant activity by using the 2,2-diphenyl-1-picrylhydrazyl radical (DPPH) assay. The antioxidant activity showed the maximum activity in the third fraction with a half-maximal inhibitory concentration (IC_{50}) value of 131.66 ppm. The fifth fragment with the IC_{50} was about 184.62 ppm. Since, the first, second, fourth, sixth, and seventh fragment were relatively weak with the IC_{50} more than 200 ppm. They were also found vitamin C 4.14 mg/100g.

Jasmina M. Zdravkovic *et al.* (2014) was conducted an experiment on antioxidant activity of three lettuce varieties (*Lactuca sativa* L.) Emerald, Vera and Neva, cultivated in two kinds of protected spaces, a glasshouse and a plastic greenhouse, under controlled conditions, was determined. The amount of antioxidant compounds: total phenols, flavonoids, L-ascorbic acid, β-carotene and lycopene, were determinate in ethanolic extracts of the lettuce with spectrophotometric methods. The maximum amount of total phenols (78.98±0.67 mg GAE/g of dry extract) was found in ethanolic extract of the lettuce variety Neva cultivated in a plastic greenhouse, whereas the maximum amount of flavonoids (35.45±0.95 mg RU/g of dry extract) was displayed in the lettuce Emerald cultivated in a glasshouse. It was noticed that the lettuce cultivated in the glasshouse contained a somewhat maximum quantity of L-ascorbic acid than the lettuce same variety from plastic greenhouse.

Ryder and Whitaker (1976) observed that lettuce is more in vitamin A and minerals like calcium and iron. It also contains protein, carbohydrate and vitamin C. There is variation in nutritive values in different types of lettuces, leaf types being the richest followed by butter heads and crisp-heads. The increased amounts are due to the increased number of green leaves exposed to light in the respective type.

Sani Ahmad Jibril *et al.* (2017) was observed that Cd stresses have adverse effect on phytochemicals and nutrient elements of lettuce when subjected to high concentrations. Antioxidants analysis which employed DPPH and FRAP, flavonoids, phenolic, vitamin C, malondialdehyde (MDA), and proline indicated significant effects of Cd treatment on the varieties tested. Different concentration levels of Cd lead to positive interactions in FRAP, phenolic, and MDA but no significant effect in flavonoids, vitamin C, and proline. The photochemical compounds bioavailability in lettuce produced is discussed in total phenolic and flavonoid, vitamin C, malondialdehyde (MDA), and proline. The significance level effects of Cd and lettuce varieties on phytochemical compounds in leaves are presented.

Shane Ardob *et al.* (2007) reported that Total phenolic content (TPC) and antioxidant capacity of lettuce were evaluated using the Folin–Ciocalteu method and DPPHd assay, respectively, on 25 cultivars of lettuce including leaf, romaine, crisp-head, and butter-head types, cultivated over two harvest periods. Leaf lettuce possessed the highest TPC and highest DPPHd scavenging ability than other cultivars. The leaf lettuce possessed the highest TPC, with a level of 43.2 mg GAE/g dry vegetable. When separated by dominant leaf color, red pigmented lettuce (52.4 mg GAE/g dry mass) possessed higher (Po0:05) TPC than green (21.7 mg GAE/g dry mass), reflecting a 2.4-fold difference. Although the red lettuce was cultivars always had higher TPC than green ones. Consistent with the assay of TPC, DPPHd scavenging mobility of red lettuce (quenched 77.9% radicals in the system) was significantly higher (Po0:05) than green lettuce (quenched 65.5% radicals in the system), reflecting a 0.8-fold difference.

Swati Saha *et al.* (2016) has reported that leaves of lettuce (*Lactuca sativa* L.) are the store house of various phytonutrients which have protective properties. In the investigation, 36 genotypes were analysed for phytochemicals such as total carotenoids, lycopene, ascorbic acid, total phenolic content, Cupric ion Reducing Antioxidant Capacity (CUPRAC) and Ferric Reducing Antioxidant Power (FRAP). Total phenolics ranged from 41.94 to 501.88 μg gallic acid/g fresh weight. The phenolic compounds have contributed significantly to the antioxidant activity in lettuce. Lettuce also provides some amount of vitamin C, calcium, iron and copper, with vitamins and minerals largely found in the leaf. Fresh lettuce leaves has good amounts of vitamin C and its intake will help in developing resistance against infectious agents and scavenge harmful pro-inflammatory free radicals.

Weiwei Zhou *et al*. (2019) was studying an observed the potential relationship between the alteration of phenolic compounds in lettuce induced by reduced nitrogen supply and its anti-proliferative effects on Caco-2 colorectal cancer cells. Our results showed that phenolic extracts from lettuce grown under low nitrogen conditions (LP) exhibited better anti-proliferative effects against Caco-2 cells, in part, by interfering with the cell cycle and inducing apoptosis, compared with those from lettuce supplied with adequate nitrogen. High performance liquid chromatography (HPLC) analysis and correlation analysis indicated that the better anticancer activity of LP may be not only related to the increased phenolic content, but also associated with the increased percentage contribution of quercetin to total phenolics. The lettuce plants contained higher total phenolic and flavonoid contents, which increased by 100.50% and 153.06%, respectively, at the end of the experiment compared to the control (CK).

Zienab F.R. Ahmed *et al.* (2021) was conducted an experiment with nutrient solution executed from Fish Waste vs. Inorganic. Organic fresh products are appreciated and are being a good fame regarding human health and environmental relation. The results transpired that the in total growth and fresh biomass of the organic NS grown lettuce were comparatively lower than those of the inorganic NS. The whole chlorophyll, carotene, phenolic compounds, and flavonoid contents, as well as antioxidant activity were significantly higher in lettuce grown in organic NS comparable to the inorganic one. Plants grown in the organic solution had lower IC_{50} value (153.1 ug/mL) which means they had significantly higher antioxidant activity comparable to inorganic plants. Higher levels of phenolic, flavonoids, chlorophyll, and carotenoids in the plant were mostly attached with antioxidant activity, which is correlative with the findings.

CHAPTER 3

MATERIALS AND METHODS

A pot experiment was carried out in the rooftop garden of Agricultural Botany department of Sher-e-Bangla Agricultural University, Dhaka, Bangladesh during the period from November 2019 to January 2020 to study the effects of organic and inorganic fertilizers on growth, yield and antioxidant properties of lettuce. The materials and methods that were used for conducting the experiment are presented under the following headings:

3.1 Experimental site: The rooftop garden of Agricultural Botany department of Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207 (Appendix I).

3.2 Weather condition:

The weather condition of crop growing period was as usual. The mean monthly air temperature during the cultivation period varied between 16.10^{o} in December and 17.75^{o} in February. Maximum and minimum relative humidity during crop growing period was 56.75 to 46.20%, respectively. Only in November 14.40 mm rainfall was recorded by Bangladesh Meteorological Department (Climate division) (Appendix- II), Agargaon, Dhaka.

3.3 Experimental materials: Experimental materials such as lettuce seedling (variety-green leaf) pot, inorganic fertilizers, cowdung, vermincompost, biochar and different chemicals for chemical analysis of lettuce were used for conducting experiment.

3.4 Collection of soil, cowdung, vermicompost and biochar:

The soil and cowdung were purchase from Amin bazaar, Dhaka. The vermicompost was collected from a nursery Ayub Agro. The biochar was collected from Bangladesh Biochar Initiative (BBI), Dhaka, Bangladesh.

3.5 Chemical analysis of soil, cowdung, vermicompost and boichar

The chemical analytical results of soil, cowdung, vermicompost and biochar were shown in Table-1 which was done at Soil Resource Development Institute (SRDI), Dhaka and Table-2 showed the content of biochar.

Table-1 Chemical analysis of soil, cowdung and vermicompost

Soil	Cowdung	Vermicompost
pH: 6.0	Moisture: 44.5%	Moisture: 53.80%
Organic matter: 1.21%	pH: 6.7	pH: 7.1
Total nitrogen: 0.061%	Organic carbon: 10.2%	Organic carbon: 10.7%
Potassium: 0.19 meq/100g	Total nitrogen: 0.65%	Total nitrogen: 1.12%
Phosphorus: 1.31 ppm	Phosphorus: 0.39	Phosphorus: 0.67%
Sulphur: 42.13 ppm	Potassium: 0.40%	Potassium: 0.95%
Zinc: o.95	Sulphur: 0.02	Sulphur: 0.01%
	Boron: 0.02%	Boron: 0.007%
	Iron: 0.003%	Iron: 0.01%
	Manganese: 0.006%	Manganese: 0.004%
	Zinc: 0.01%	Zinc: 0.01%
	Copper: 0.002%	Copper: 0.003%
	Chromium: 10.12 ppm	Chromium: 22.43 ppm
	Cadmium: 0.19 ppm	Cadmium: 0.44 ppm
	Lead: 5.76 ppm	Lead: 2.97 ppm

Source: SRDI

Table-2 Chemical content of biochar

Nutrients	Percent amount
Organic matter	>50%
Total nitrogen (N)	4.00%
Phosphorus pentoxide	2.00%
Potassium (K)	4.00%
Amino acid	>3%
Humic acid	>6%

Source: BBI

3.6 Crop: The lettuce variety of green leaf seedling was collected from Folbithi Horticultural Center, Asadgate, Dhaka.

3.7 Experiment design:

A single factor experiment with twelve treatments was carried out in Completely Randomized Design (CRD) with four (4) replications.

3.8 Preparation of pot soil:

According to the treatment pots were filled with prepared soil media. Inorganic fertilizers was used as the recommendation of Fertilizer Recommendation Guide 2018.

3.9 Treatments of the experiment:

$\mathbf{T_o}$ Soil 100%

$\mathbf{T_1}$ Soil 100% + Inorganic fertilizer

$\mathbf{T_2}$ Soil 80% + Cowdung 20%

$\mathbf{T_3}$ Soil 90% + Vermicompost 10%

$\mathbf{T_4}$ Soil 95% + Biochar 5%

$\mathbf{T_5}$ Soil 80% + Cowdung 20% + Inorganic fertilizer

$\mathbf{T_6}$ Soil 90% + Vermicompost 10% + Inorganic fertilizer

T_7 Soil 95% + Biochar 5% + Inorganic fertilizer

T_8 Soil 70% + Cowdung 20% + Vermicompost 10% + Inorganic fertilizer

T_9 Soil 75% +Cowdung 20% + Biochar 5% + Inorganic fertilizer

T_{10} Soil 85%+ Vermicompost 10% + Biochar 5% + Inorganic fertilizer

T_{11} Soil 65% + Cowdung 20% + Vermicompost 10% + Biochar 5% + Inorganic fertilizer

3.10 Date of transplanting: The lettuce seedling was transplanted in the plot an 23 November 2019.

3.11 Intercultural operations

After transplanting the seedlings, different kinds of intercultural operations were completed for better growth and development of the plants, which are as follows:

3.11.1 Weeding

When weeds need to be cleaned from the crop then weeding is done for break the crust and better soil aeration.

3.11.2 Irrigation

Light irrigation was given immediately after transplanting the seedlings and it was continued to the seedlings established in the field. Then irrigation was provided as required.

3.11.3 Staking

Staking was given to each plant by bamboo sticks to keep them vertical.

3.12 Date of harvest: The all lettuce shoot were harvested on 3 January 2020. Necessary data were recorded.

3.13 Data Collection and Recording: The following parameters were recorded during the study:

1. Plant height (cm)
2. Number of leaves per plant
3. Shoot fresh weight (g)
4. Shoot dry weight (g)
5. Root length (cm)
6. Root fresh weight (g)
7. Root dry weight (g)
8. Vitamin C
9. Antioxidant activity with IC_{50} value
10. Phenolic compound
11. Flavonoid content

3.14 Procedure of recording data

3.14.1 Plant height (cm)

Plant height was recorded at 15, 25 and 35 days after transplanting and harvest. The height of the plant was determined in centimeter (cm).

3.14.2 Leaf number

Leaf number was recorded at 15, 25 and 35 days after transplanting and harvest. The number of leaves plant^{-1} was counted from each plant.

3.14.3 Shoot fresh weight (g)

Total leaf fresh weight plant^{-1} was measured using an electrical balance in gram (g)

3.14.4 Shoot dry weight (g)

Total leaf dry weight plant^{-1} was measured using an electrical balance in gram (g)

3.14.5 Root length (cm) Root length was recorded when it was harvested. Thus value was recorded and expressed in centimeter (cm).

3.14.6 Root fresh weight (g)

Total root fresh weight per plant will be measured using an electrical balance in gram

3.14.7 Root dry weight (g)

Total root dry weight per plant will be measured using an electrical balance in gram

3.14.8 Vitamin C Test

a) Equipment needed

1. Burette and stand
2. 250 ml
3. 20 ml pipette
4. 250 ml conical flask
5. Measuring cylinder
6. Filter paper

b) Plant material

Field experiments as part of this study were conducted in the winter cycle of lettuce cultivation in the 2019-2020 growing season. The experiments were performed on Sher-e-Bangla Agricultural University. The lettuce was produced from seedlings planted. When plant mature then the plant sample collected and washed in distil water then use blotting paper for absorbed water from plant sample. Plant samples were wrapped with aluminium foil paper and stored at -10^0C in the refrigerator.

c) Analytical procedures

Extraction of the plant material

Total vitamin C was extracted from the measured quantity of fresh lettuce (100g) by using the mixture of meta-phosphoric acid (HPO_3) and glacial acetic acid (CH_3COOH). The gained extract was filtrated through filter paper, and it taken for titration with Tillman's reagent.

Tillman's method

Quantitative determination of L-ascorbic acid is depending on the reversible capability of oxido-reduction system of ascorbic-de-hydro-ascorbic acid. Titration with the reagent 2, 6-dichlorophenolindophenol (Tillman's reagent, TR) was completed in an acidic environment with pH of 4 to 6. The oxidized form of TR (also serving as an indicator) is dark blue at pH of 5.2. In the presence of ascorbic acid, TR changes into its reduced colourless form. At pH of 4.2 TR is red (acidic environment), and when the whole amount of L-ascorbic acid is oxidized, the very next drop of TR colours the tested solution pink.

Preparation of the reagent's solution

A total of 0.26 g of 2,6-dichlorophenolindophenol was solved in 1L of warm distilled water containing 0.21 g of $NaHCO_3$. Upon dissolution, the container was filled with up to 1 dm^3 of freshly boiled cooled distilled water. The solution was subsequently filtrated through filter paper and kept in a dark bottle in the refrigerator. The solution was stable for up to four weeks. Before use, the titter of TR was determined according to standard 1mg/cm^3 ascorbic acid solution.

L-ascorbic acid determination procedure

Three parallel titrations were performed for each sample. The determined volume of the lettuce extract (5.00 ml of extract + 3% metaphosphoric acid (HPO_3)) was titrated with Tillman's reagent until it turned into light pink colour that persisted for about five seconds.

3.14.9 Antioxidant test

a) **Drying and Grinding**

The collected plant samples were separated from undesired materials. Then it was dried in the sun for one week after cutting into small pieces. The plant parts were ground into coarse powder with the help of a suitable grinder. The powder was stored in an airtight container and kept in a cool, dark and dry place until analysis complete.

b) Preparation of Plant Extract

The powdered sample was taken in a clean, flat-bottomed glass container and soaked in 90% methanol. The container with its contents was sealed and kept for a period of 15 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by apiece of clean, white cotton material. Then it was filtered through whatman filter paper. The filtrate was kept in an open space to evaporate the solvent thus crude extract was obtained. Fine powders of the lettuce leaves are dissolved in 90% methanol and then the solvent are evaporate.

c) Antioxidant activity with IC_{50} value

Stock solution of the plant extract was prepared in methanol (10mg/ml) from which a serial dilution was carried out (Valco et al., 2004; Clarkson, 1995; Mohammad Moniruzzaman 2020). At first 6 volumetric flasks were taken to make 6 different types of concentration 1, 5, 10, 50, 100 and 500 μg/ml. Test tubes and volumetric flasks were covered with foil paper. In 6 volumetric flasks serial rank of extract was done and numbered them respectively. 2ml of sample from each concentration and 2 ml of 0.004% DPPH (2, 2-diphenyl-1-picrylhydrazyl) solution was taken with the help of pipette in 6 test tubes respectively. Then solution was kept in dark place for 35 minutes with covering every test tube with foil paper. In another test tube 2ml 0.004% DPPH & 2ml methanol was taken for the prepare blank solution. Then absorbance is taken by UV Spectroscopy. The percent of inhibition was calculated by applying following equation-

$$\%\ \text{Inhibition} = \frac{\text{Blank absorbance} - \text{Solution absorbance}}{\text{Blank absorbance}} \times 100$$

3.14.10 Total Phenolic Content

The total phenolic content of the extract was measured by the modified Folin-Ciocalteu's method (Zilani M.N et al., 2016). Methanol solution of the extract (1mg/mL) was mixed with 5mL of 10% (*v*/v) Folin-Ciocalteu reagent. Then 4mL sodium carbonate (75g/L) was added to the mixture. It was kept at 40°C for 30 min. Absorbance of the reaction mixture was assessment at 765 nm. Different concentrations (0.1–0.5 mg/ mL) of gallic acid was used to prepare the standard calibration curve from where total phenol content was determinate and exposed as mg gallic acid equivalent GAE/gm of dry extract.

3.14.11 Total Flavonoids Content

Total flavonoid quantity was estimated using aluminium chloride colorimetric assay (Mahmud I. et al. 2017). In 1mL of the extract solution (1mg/ml), 0.2mL aluminium chloride (1% *w*/*v*), 0.2 ml potassium acetate (1 M) and 5.4 ml distilled water were assimilated and mixed well. Then absorbance was measurable at 415nm against blank solution. For this assay, quercetin (0.1–0.5mg/mL) was used to prepare standard calibration curve and total flavonoid content of the extract was exposed in terms of mg quercetine equivalent QE/gm of dried extract.

3.15 Statistical analysis

All the data collected on different parameters were statistically analyzed following the analysis of variance (ANOVA) technique using MSTAT software and the mean differences were adjudged by least significant difference (LSD) test at 5% level of significance.

CHAPTER 4

RESULTS AND DISCUSSION

The present experiment was carried to determine the effect of different fertilizer on growth and yield of lettuce. Data on different growth parameters after transplanting (DAT) and yield of lettuce plant were recorded and analyzed with *Statistix 10* software. The results have been submitted and discussed, and possible explanations are given under the following headings:

4.1 Plant height

Plant height (cm) of lettuce differed significantly at 25 and 35 days after transplanting (DAT) due to the application of different organic and inorganic fertilizer (Table 3, Appendix-III). At 25 DAT, tallest plant height (13.10 cm) was observed from the treatment T_3 (Soil 90% + Vermicompost 10%) while the shortest plant height (10.10 cm) was found from control (T_0) (Soil 100%). At 35 DAT, the maximum plant height (23.85 cm) was found from the treatment T_3 (Soil 90% + Vermicompost 10%) which statistically similar with 22.80 cm, 22.65 cm and 22.55 cm from the treatment T_2 (Soil 80% + Cowdung 20%), T_{11} (Soil 65% + Cowdung 20% + Vermicompost 10% + Biochar 5% + Inorganic fertilizer) and T_4 (Soil 95% + Biochar 5%), respectively. The shortest (19.80 cm) was recorded from the control (T_0) (Soil 100%) at 35 DAT. In the present study the highest plant height was obtained while the plants treated with organic fertilizers such as cowdung, vermicompost and biochar. At 15 DAT, the plant did not show any significant difference among the treatments. The highest plant height (7.20 cm) was recorded from the treatment of T_3 (Soil 90% + Vermicompost 10%) and the treatment T_1 (Soil 100% + Inorganic fertilizer) showed the lowest plant height (6.40 cm). Zaman *et al.,* (2017) reported that the application of cowdung influenced on *Stevia rebaudiana* plant height and it was increased with the advanced doses of cowdung. The results of plant height of this study were consistent with finding of Kanwar *et al*. (2002) and Barani and Anburani (2004) who stated that vermicompost showed better results on cauliflower and bhendi than other organic fertilizers. Amendola *et al*. (2017) reported that together application of biochar and compost increased the plant height in the crops of grapevine. These results indicate that organic manure increases the growth of lettuce which ensured the tallest plant height than control. Therefore all together, it suggests that organic fertilizers such as cowdung, vermicompost and biochar increase the plant height of lettuce either in sole or together application organic and inorganic fertilizers.

Table-3 Effect of inorganic and organic fertilizer on plant height of lettuce at different days after transplanting (DAT).

Treatment	Plant height (cm) at different days after transplanting (DAT)		
	15 DAT	25 DAT	35 DAT
T_0	6.60	10.10 e	19.80 c
T_1	6.40	10.20 de	20.10 c
T_2	6.85	11.85 bc	22.80 ab
T_3	7.20	13.10 a	23.85 a
T_4	6.90	12.10 ab	22.55 ab
T_5	6.60	11.90 bc	20.70 bc
T_6	6.80	11.83 bc	21.80 abc
T_7	6.95	11.20 cde	21.05 bc
T_8	6.65	11. 30 bcd	21.70 abc
T_9	6.90	11.60 bc	21.60 abc
T_{10}	6.60	11.60 bc	22.15 abc
T_{11}	6.75	11.70 bc	22.65 ab
LSD (0.05)	0.68	1.16	2.45
Significant Level	NS	*	*
CV%	7.04	7.01	7.86

In a column, means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly as per 0.05 level of probability.

T_0 = Soil 100% , T_1= Soil 100% + Inorganic fertilizer , T_2 = Soil 80% + Cowdung 20% , T_3 = Soil 90% + Vermicompost 10% , T_4 = Soil 95% + Biochar 5% , T_5 = Soil 80% + Cow dung 20% + Inorganic fertilizer, T_6 = Soil 90% + Vermicompost 10% + Inorganic fertilizer, T_7= Soil 95% + Biochar 5% + Inorganic fertilizer, T_8 = Soil 70% + Cow dung 20% + Vermicompost 10% + Inorganic fertilizer, T_9 = Soil 75% +Cow dung 20% + Biochar 5% + Inorganic fertilizer, T_{10} = Soil 85%+ Vermicompost 10% + Biochar 5% + Inorganic fertilizer, T_{11} = Soil 65% + Cowdung 20% + Vermicompost 10% + Biochar 5% + Inorganic fertilizer.

4.2 Number of leaves plant^{-1}

Number of leaves plant^{-1} was significantly influenced by organic fertilizer (Table-4, Appendix-IV). At 15 DAT the maximum leaf number 8.50 was recorded from the treatment T_3 (Soil 90% + Vermicompost) but the minimum leaf number 6.00 was found from the control (T_0). The maximum 17.75 leaf number was observed from the treatment T_4 (Soil 95% + Biochar 5%) and the minimum 12.00 was recorded from the control (T_0) at 25 DAT. The highest 24.25 leaf number was observed from the treatment T_3 (Soil 90% + Vermicompost 10%) which is statistically similar 23.75 from the treatment T_{11} (Soil 65% + Cowdung 20% + Vermicompost 10% + Biochar 5% + Inorganic fertilizer) and the minimum 18.50 was found from the control (T_0) at 35 DAT. Cowdung, vermicompost and biochar application confirmed that the availability of essentials nutrients for plant and the final results is the highest number of leaves per plant than control condition. Number of leaves increased with sole or together application of cowdung, vermicompost and biochar in soil than control (T_0). Organic manure supplied available essential nutrients to the plant and produced the highest number of leaves per plant compare to control condition. The results of number of leaf per plant of this study were consistent with finding of Masarirambi *et al.* (2010) and Shane Ardob *et al.* (2007) who stated that organic fertilizers showed better results of red lettuce. Adriana Hernández *et al.* (2010) reported that vermicompost more influenced increase leaves number than other organic fertilizers. Therefore, all together it suggests that sole or combined application of organic and inorganic fertilizers increased leaf number in lettuce plant.

Table-4 Effect of inorganic and organic fertilizer on number of leaves plant^{-1} of lettuce at different days after transplanting (DAT).

Treatment	Leaf Number plant^{-1}		
	15 DAT	**25 DAT**	**35 DAT**
T_o	6.00 d	12.00 e	18.50 e
T_1	6.25 d	12.25 de	18.75 e
T_2	8.25 ab	17.00 ab	22.00 abc
T_3	8.50 a	17.50 ab	24.25 a
T_4	7.75 abc	17.75 a	22.25 abc
T_5	6.50 cd	13.50 cde	19.25 de
T_6	6.75 cd	12.50 de	20.25 cde
T_7	6.50 cd	13.25 cde	20.75 cde
T_8	6.75 cd	14.25 cde	21.50 bcd
T_9	6.75 cd	14.50 cd	21.75 bc
T_{10}	7.00 bcd	15.25 bc	21.75 bc
T_{11}	8.20 ab	15.25 bc	23.75 ab
LSD (0.05)	1.44	2.29	2.45
Significant Level	*	*	*
CV%	14.29	10.99	8.04

In a column, means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly as per 0.05 level of probability.

T_0 = Soil 100% , T_1= Soil 100% + Inorganic fertilizer , T_2 = Soil 80% + Cowdung 20% , T_3 = Soil 90% + Vermicompost 10% , T_4 = Soil 95% + Biochar 5% , T_5 = Soil 80% + Cow dung 20% + Inorganic fertilizer, T_6 = Soil 90% + Vermicompost 10% + Inorganic fertilizer, T_7= Soil 95% + Biochar 5% + Inorganic fertilizer, T_8 = Soil 70% + Cow dung 20% + Vermicompost 10% + Inorganic fertilizer, T_9 = Soil 75% +Cow dung 20% + Biochar 5% + Inorganic fertilizer, T_{10} = Soil 85%+ Vermicompost 10% + Biochar 5% + Inorganic fertilizer, T_{11} = Soil 65% + Cowdung 20% + Vermicompost 10% + Biochar 5% + Inorganic fertilizer.

4.3 Shoot fresh weight

Organic fertilizer significantly influenced the fresh weight of shoot of each plant. Different levels of the treatments showed significant difference of shoot fresh weight (Table 5, Appendix V). The maximum shoot fresh weight 104.85 g was recorded from T_3 (Soil 90% + Vermicompost 10%) which is statistically similar 104.07 g from the treatment of T_{11} (Soil 65% + Cowdung 20% + Vermicompost 10% + Biochar 5% + Inorganic fertilizer). The lowest shoot fresh weight 82.23 g was found from the control. These results are consistent with the results of plant height (Table 3) and leaf number (Table 4) of lettuce of this study. A significant variation was found due to together use of organic and inorganic fertilizers. Among the different organic fertilizers Vermicompost was more effective than other organic fertilizers. Organic substances have a positive effect on fresh weight of lettuce. These results are supported by the finding of Huang and Tsai (1993) who reported that combined effect of organic and inorganic fertilizers showed better performance in lettuce crop than of other inorganic fertilizers. Villas B. *et al*. (2004) reported that organic fertilizers influenced the shoot growth in lettuce crops. Therefore all together it suggests that shoot fresh weight increase with the sole or combined application of inorganic fertilizers.

4.4 Shoot dry weight:

Organic manure significantly influenced the dry weight of shoot as fresh eight of lettuce. The maximum dry weight 12.68 g was observed from the treatment T_{11} (Soil 65% + Cowdung 20% + Vermicompost 10% + Biochar 5% + Inorganic fertilizer) which is nearest value 12.50 g, 12.45 g was found from the treatment T_3 (Soil 90% + Vermicompost) and T_2 (Soil 80% + Cowdung 20%). The minimum dry weight 5.95 g was recorded from the control (Table 5, Appendix V). The results of shoot dry weight of this study were almost similar trend with the plant height, leaf number and shoot fresh weight. Therefore, it suggests that dry weight shoot of lettuce increase with sole or combined application of cowdung, vermicompost and biochar in media in the rooftop garden. Sharma *et al*. (2001) reported that shoot dry weight was maximum in organic fertilizers as compared to inorganic fertilizers which are support in this study.

Table-5 Effect of inorganic and organic fertilizer on shoot fresh weight and shoot dry weight of lettuce plant.

Treatment	Shoot fresh weight (g)	Shoot dry weight (g)
T_o	82.23 e	5.95 e
T_1	86.15 de	7.20 e
T_2	103.25 abc	12.45 ab
T_3	104.85 a	12.50 a
T_4	102.50 abc	12.25 abc
T_5	94.18 cd	10.65 d
T_6	93.73 cd	10.93 bcd
T_7	94.83 bcd	10.83 cd
T_8	97.55 abc	11.15 abcd
T_9	98.18 abc	11.33 abcd
T_{10}	101.53 abc	11.78 abcd
T_{11}	104.07 ab	12.68 a
$LSD_{(0.05)}$	9.74	1.54
Significant Level	*	*
CV%	7.01	9.96

In a column, means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly as per 0.05 level of probability.

T_0 = Soil 100% , T_1= Soil 100% + Inorganic fertilizer , T_2 = Soil 80% + Cowdung 20% , T_3 = Soil 90% + Vermicompost 10% , T_4 = Soil 95% + Biochar 5% , T_5 = Soil 80% + Cow dung 20% + Inorganic fertilizer, T_6 = Soil 90% + Vermicompost 10% + Inorganic fertilizer, T_7= Soil 95% + Biochar 5% + Inorganic fertilizer, T_8 = Soil 70% + Cow dung 20% + Vermicompost 10% + Inorganic fertilizer, T_9 = Soil 75% +Cow dung 20% + Biochar 5% + Inorganic fertilizer, T_{10} = Soil 85%+ Vermicompost 10% + Biochar 5% + Inorganic fertilizer, T_{11} = Soil 65% + Cowdung 20% + Vermicompost 10% + Biochar 5% + Inorganic fertilizer.

4.5 Root length

Root length of lettuce was increased significantly due to various treatments of addition of organic and inorganic fertilizers in soil media. Root length (cm) differed significantly because of the application of different organic and inorganic fertilizer (Table 6, Appendix VI). The highest root length 15.37 cm was recorded from Treatment of T_{11} and the statistically nearest value 15.13 cm and 14.88 cm found from the treatment T_9 (Soil 75% + Cowdung 20% + Biochar 5% + Inorganic fertilizer) and T_{10} (Soil 85% + Vermicompost 10% + Biochar 5% + Inorganic fertilizer). But the lowest value 11.50 cm was found from the control (T_O) which was statistically similar with T_1 (11.67 cm). These results suggest that the root length of lettuce increased at higher rate in together application of organic and inorganic fertilizers but sole application of either inorganic or organic fertilizers did not response as combined application of organic and inorganic fertilizers.

4.6 Root fresh weight

Root fresh weight of lettuce significantly increased with the different treatment of this study (Table 6, Appendix VI). The highest root fresh weight (12.47 g) of lettuce was found from the treatment of T_{11} (Soil 65% + Cowdung 20% + Vermicompost 10% + Biochar 5% + Inorganic fertilizer) and the lowest value (8.57 g) was found from the control (T_o) treatment. These data are also constant with the morphological previous parameter including plant height, leaf number and root length of this study. Therefore, it suggest that root fresh weight of lettuce increased as much high with the combined application of organic and inorganic fertilizers than sole application of organic or inorganic fertilizers in the soil media.

4.7 Root dry weigh

Root dry weight of lettuce significantly increased with the different treatment of this study (Table 6, Appendix VI). The highest root dry weight (1.19 g) of lettuce was recorded from the treatment of T_{11} (Soil 65% + Cowdung 20% + Vermicompost 10% + Biochar 5% + Inorganic fertilizer) and the lowest value (0.84 g) was found from the control (T_o). These data are also consistent with the morphological previous parameter including, plant height, leaf number, root length and root fresh weight of this study. Therefore, it suggest that root dry weight of lettuce increased as much high with the together application of organic and inorganic fertilizers than sole application of organic or inorganic fertilizers in the soil media.

Table-6 Effect of inorganic and organic fertilizer of root length, root fresh weight and root dry weight of lettuce.

Treatment	Root length (cm)	Root fresh weight (g)	Root dry weight (g)
T_o	11.50 e	8.57 f	0.84 f
T_1	11.67 e	9.25 ef	0.87 ef
T_2	13.12 cd	10.04 def	0.96 de
T_3	12.55 de	10.66 bcde	0.98 de
T_4	13.30 cd	10.28 cde	0.97 de
T_5	13.95 bc	11.03 abcd	1.01 cd
T_6	13.34 cd	10.78 bcde	1.01 cd
T_7	13.94 bc	11.68 abc	0.99 d
T_8	14.36 abc	11.76 abc	1.05 bcd
T_9	15.13 ab	11.57 abcd	1.12 abc
T_{10}	14.88 ab	12.01 ab	1.14 ab
T_{11}	15.37 a	12.47 a	1.19 a
LSD (0.05)	1.38	1.58	0.11
Significant Level	*	*	*
CV%	7.08	10.13	7.77

In a column, means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly as per 0.05 level of probability.

T_0 = Soil 100%, T_1= Soil 100% + Inorganic fertilizer, T_2 = Soil 80% + Cowdung 20% , T_3 = Soil 90% + Vermicompost 10% , T_4 = Soil 95% + Biochar 5% , T_5 = Soil 80% + Cow dung 20% + Inorganic fertilizer, T_6 = Soil 90% + Vermicompost 10% + Inorganic fertilizer, T_7= Soil 95% + Biochar 5%, T_8 = Soil 70% + Cow dung 20% + Vermicompost 10% + Inorganic fertilizer, T_9 = Soil 75% +Cow dung 20% + Biochar 5% + Inorganic fertilizer, T_{10} = Soil 85%+ Vermicompost 10% + Biochar 5% + Inorganic fertilizer, T_{11} = Soil 65% + Cowdung 20% + Vermicompost 10% + Biochar 5% + Inorganic fertilizer.

4.8 Antioxidant activity and antioxidant compound

4.8.1 Antioxidants test on DPPH scavenging assay:

DPPH radical scavenging activity assay is one of the greatest workable methods for screening the antioxidant activity of plant extracts. The result of DPPH free radical scavenging activity of methanol extract from lettuce leaf was measured as IC_{50} value estimation. The results of IC_{50} value showed significant differences among the different treatments of this study (Table 7, Appendix VII). The results were compared significantly active against standard ascorbic acid. Herpanti et al. (2021) reported that lower IC_{50} value which means they had significantly higher antioxidant activity. In the present study, the result for DPPH radical scavenging activity is presented as IC_{50} values. As shown under the Table-7, highly antioxidant activities with the half-maximum inhibitory concentration (IC_{50}) was 83.01μg/ml found from the treatments T_3 (Soil 90% + Vermicompost 10%). T_2 (Soil 80% + Cowdung 20%,) and T_4 (Soil 80% + Cowdung 20%,) showed statistically nearest IC_{50} values. In these experimental results showed that organic fertilizer show lowest IC_{50} value compared to the inorganic fertilizer. Therefore, it suggest that application of inorganic fertilizers increased IC_{50} value which mean lower antioxidant activity with inorganic fertilizers, while the addition of organic fertilizers decrease the IC_{50} value which mean higher antioxidant activity. These results are supported by many previous finding (Chang Ha Park et al. 2018).

Table-7 Antioxidant activity (IC_{50}) of lettuce in organic and inorganic fertilizers.

Treatment	IC_{50} (µg/ml)
T_o	120.64 b
T_1	137.56 a
T_2	88.36 de
T_3	83.01 e
T_4	87.60 de
T_5	106.82 bc
T_6	101.28 cd
T_7	103.44 c
T_8	96.99 cde
T_9	96.66 cde
T_{10}	96.49 cde
T_{11}	93.71 cde
LSD (0.05)	14.67
Significant Level	*
CV%	10.13

Standard ascorbic acid IC_{50} value found 6.14 (µg/ml).

In a column, means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly as per 0.05 level of probability using the LSD test.

T_0 = Soil 100%, T_1= Soil 100% + Inorganic fertilizer, T_2 = Soil 80% + Cowdung 20%, T_3 = Soil 90% + Vermicompost 10% , T_4 = Soil 95% + Biochar 5% , T_{10} = Soil 85%+ Vermicompost 10% + Biochar 5% + Inorganic fertilizer, T_5 = Soil 80% + Cow dung 20% + Inorganic fertilizer, T_6 = Soil 90% + Vermicompost 10% + Inorganic fertilizer, T_7= Soil 95% + Biochar 5% + Inorganic fertilizer, T_8 = Soil 70% + Cow dung 20% + Vermicompost 10% + Inorganic fertilizer, T_9 = Soil 75% +Cow dung 20% + Biochar 5% + Inorganic fertilizer, T_{11} = Soil 65% + Cowdung 20% + Vermicompost 10% + Biochar 5% + Inorganic fertilizer.

4.8.2 Vitamin C:

The highest vitamin C value 4.33 mg/100g was found from the treatment T_3 (Soil 90% + Vermicompost 10%) and statistically nearest value 4.23 mg/100g and 4.22 mg/100g found from the treatment T_2 (Soil 80% + Cowdung 20%) and T_4 (Soil 95% + Biochar 5%). The lowest value 3.12 mg/100g found from the treatment T_1 (Soil 100% + Inorganic fertilizer). Vitamin C level significantly decreased in inorganic fertilizers compared with the organic fertilizers. Vitamin C increased with sole or combined application of cowdung, vermicompost and biochar in soil than control (T_0). Therefore it suggests that vitamin C content is dependent upon the soil organic and inorganic fertilizers. Herpandi et al. (2021), Lairon et al. (1984) and Schuphan (1974) reported that lettuce grown organically were highest in ascorbic acid compared to those grown conventionally. These results are consistent with this experiment. In this experiment measured vitamin C content of lettuce leaf was as antioxidant compound. The amount of vitamin C showed significant different to the different treatments of this study (Table 8, Appendix VIII).

Table-8 Effect of inorganic and organic fertilizer of Vitamin C in lettuce leaves.

Treatment	Vitamin C (mg/100g)
T_o	3.23 c
T_1	3.17 c
T_2	4.23 a
T_3	4.33 a
T_4	4.22 a
T_5	3.56 bc
T_6	3.66 bc
T_7	3.67 bc
T_8	3.88 ab
T_9	3.89 ab
T_{10}	3.97 ab
T_{11}	4.04 ab
LSD (0.05)	0.55
Significant Level	*
CV%	10.09

In a column, means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly as per 0.05 level of probability using the LSD test.

T_0 = Soil 100%, T_1= Soil 100% + Inorganic fertilizer , T_2 = Soil 80% + Cowdung 20%, T_3 = Soil 90% + Vermicompost 10% , T_4 = Soil 80% + Cowdung 20%, T_5 = Soil 80% + Cow dung 20% + Inorganic fertilizer, T_6 = Soil 90% + Vermicompost 10% + Inorganic fertilizer, T_7= Soil 95% + Biochar 5% + Inorganic fertilizer, T_8 = Soil 70% + Cow dung 20% + Vermicompost 10% + Inorganic fertilizer, T_9 = Soil 75% +Cow dung 20% + Biochar 5% + Inorganic fertilizer, T_{10} = Soil 85%+ Vermicompost 10% + Biochar 5% + Inorganic fertilizer, T_{11} = Soil 65% + Cowdung 20% + Vermicompost 10% + Biochar 5% + Inorganic fertilizer.

4.8.3 Total phenolic content

Total phenolic content (TPC) of selected twelve treatment of lettuce ranged from 53.71 to 87.68 mg gallic acid equivalent per gram dry weight (mg GAE/g DW). Here, total phenolic content, the absorbance values received in this test using the different concentrations of gallic acid were decorated against respective concentrations. Total phenolic content of the lettuce leaves extract was calculated using this equation. Statistical analysis of these results showed significant differences (Table 9, Appendix- IX). In the studies highest phenolic contents 87.68 mg GAE/g DW was recorded from the treatment T_3 (Soil 90% + Vermicompost 10%) which was statistically similar 86.82 mg GAE/g DW and 86.12 mg GAE/g DW from T_4 (Soil 95% + Biochar 5%) and T_2 (Soil 80% + Cowdung 20%) respectively. This result indicates that maximum phenolic compound found in lettuce from organic fertilizer treatment (Table 9, Appendix X). Separately, the addition of inorganic fertilizers with growing media showed the negative effect on the content of TPC in lettuce. Kim Heon-Woong et al. (2019) and Zienab et al. (2021) reported that total phenolic content higher in organic manure compares with inorganic fertilizers in lettuce. Chang Ha Park et al. (2018) conducted an experiment and found similar trend of results of total phenolic contents of lettuce. Therefore, altogether it suggests that the application of organic fertilizers significantly improve the quality of food crops including lettuce.

Table-9 Total phenolic content of lettuce in organic and inorganic fertilizers

Treatment	Phenolic Content (GAE/gm dry extract)
T_o	64.30 b
T_1	53.71 c
T_2	86.12 a
T_3	87.68 a
T_4	86.82 a
T_5	67.07 b
T_6	67.74 b
T_7	67.63 b
T_8	63.76 bc
T_9	63.47 bc
T_{10}	62.86 bc
T_{11}	82.83 a
LSD (0.05)	10.29
Significant Level	*
CV%	10.09

In a column, means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly as per 0.05 level of probability using the LSD test.

T_0 = Soil 100%, T_1= Soil 100% + Inorganic fertilizer, T_2 = Soil 80% + Cowdung 20%, T_3 = Soil 90% + Vermicompost 10% , T_4 = Soil 95% + Biochar 5% , T_5 = Soil 80% + Cow dung 20% + Inorganic fertilizer, T_6 = Soil 90% + Vermicompost 10% + Inorganic fertilizer, T_7= Soil 95% + Biochar 5% + Inorganic fertilizer, T_8 = Soil 70% + Cow dung 20% + Vermicompost 10% + Inorganic fertilizer, T_9 = Soil 75% +Cow dung 20% + Biochar 5% + Inorganic fertilizer, T_{10} = Soil 85%+ Vermicompost 10% + Biochar 5% + Inorganic fertilizer, T_{11} = Soil 65% + Cowdung 20% + Vermicompost 10% + Biochar 5% + Inorganic fertilizer.

4.8.4 Total flavonoid content

Total flavonoid content of selected twelve treatment of lettuce ranged from 26.98 to 44.49 mg quercetin equivalent per gram dry weight (mg QE/g DW). Statistical analysis of these results showed significant differences exist among the lettuce ($p<0.05$) produced under different treatment. Here, total flavonoid content, the absorbance values received in this test using the different concentrations of quercetin equivalent were decorated against respective concentrations. In the studies highest flavonoid contents 44.49 mg QE/g DW was recorded from the treatment T_3 (Soil 90% + Vermicompost 10%) which was statistically similar 43.76 mg QE/g DW and 43.21 mg QE/g DW from T_4 (Soil 95% + Biochar 5%) and T_2 (Soil 80% + Cowdung 20%). This result was indicated that maximum flavonoid content found from organic fertilizer compared to the inorganic fertilizer (Table 10, Appendix X). Total flavonoid content increased with sole or together application of cowdung, vermicompost and biochar in soil than control (T_0). Chang Ha Park et al. (2018) was conducted an experiment compared red skirt and green lettuce plant, determined the total flavonoid contents and that results are also similar in this experiment. Separately, the addition of inorganic fertilizers with growing media showed the negative effect on the content of TFC in lettuce. Kim Heon-Woong et al. (2019) and Zienab et al. (2021) reported that total flavonoid content higher in organic manure compared with inorganic fertilizers in lettuce. Determination of the total flavonoid content (TFC) in lettuce fresh weight extract was using the aluminium chloride colorimetric method by Gan and Azrina (2016). Jasmina M. Zdravkovic et al. (2014) was found similar flavonoid content in lettuce plant.

Table-10 Total flavonoid content of lettuce in organic and inorganic fertilizers

Treatment	Flavonoid (mg QE/g)
T_o	32.03 bc
T_1	26.98 d
T_2	43.21 a
T_3	44.49 a
T_4	43.76 a
T_5	33.87 b
T_6	33.88 b
T_7	34.30 b
T_8	32.39 b
T_9	27.35 cd
T_{10}	31.89 bc
T_{11}	41.76 a
LSD (0.05)	4.87
Significant Level	*
CV%	9.57

In a column, means having similar letter(s) are statistically similar and those having dissimilar letter(s) differ significantly as per 0.05 level of probability using the LSD test.

T_0 = Soil 100% , T_1= Soil 100% + Inorganic fertilizer , T_2 = Soil 80% + Cowdung 20% , T_3 = Soil 90% + Vermicompost 10% , T_4 = Soil 95% + Biochar 5% , T_5 = Soil 80% + Cow dung 20% + Inorganic fertilizer, T_6 = Soil 90% + Vermicompost 10% + Inorganic fertilizer, T_7= Soil 95% + Biochar 5% + Inorganic fertilizer, T_8 = Soil 70% + Cow dung 20% + Vermicompost 10% + Inorganic fertilizer, T_9 = Soil 75% +Cow dung 20% + Biochar 5% + Inorganic fertilizer, T_{10} = Soil 85%+ Vermicompost 10% + Biochar 5% + Inorganic fertilizer, T_{11} = Soil 65% + Cowdung 20% + Vermicompost 10% + Biochar 5% + Inorganic fertilizer.

CHAPTER 5

SUMMERY AND CONCLUSION

The experiment was carried out during the period of November 2019 to May 2020 at the roof top garden of Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207 to find out the effect of organic and inorganic fertilizers on yield and antioxidant properties of lettuce plant. Twelve (12) treatments were included for the present study viz. T_0 = Soil 100%, T_1= Soil 100% + Inorganic fertilizer, T_2 = Soil 80% + Cowdung 20%, T_3 = Soil 90% + Vermicompost 10% , T_4 = Soil 95% + Biochar 5% , T_5 = Soil 80% + Cow dung 20% + Inorganic fertilizer, T_6 = Soil 90% + Vermicompost 10% + Inorganic fertilizer, T_7= Soil 95% + Biochar 5% + Inorganic fertilizer, T_8 = Soil 70% + Cow dung 20% + Vermicompost 10% + Inorganic fertilizer, T_9 = Soil 75% +Cow dung 20% + Biochar 5% + Inorganic fertilizer, T_{10} = Soil 85%+ Vermicompost 10% + Biochar 5% + Inorganic fertilizer, T_{11} = Soil 65% + Cowdung 20% + Vermicompost 10% + Biochar 5% + Inorganic fertilizer. The experiment was laid out in completely randomized design (CRD) with four replications. Different growth and yield parameters were recorded and statistically analyzed, also antioxidant properties were analyzed statistically. The yield and yield contributing characters were significantly influenced by different treatments. At 15 DAT the tallest (7.20 cm) plant was recorded from the treatment T_3, while the control gave the shortest (6.60 cm) plant. The tallest (13.10 cm) plant height was observed from the treatment T_4 and the shortest (10.10 cm) was found from control at 25 DAT. The maximum plant height (23.85 cm) was recorded from the treatment T_3 and the lowest value (19.80 cm) was found from the control. Also the maximum leaf number per plant (8.50, 17.75 and 24.25 respectively) from the treatment T_3, T_4 and T_3 and the minimum leaf number per plant (6.00, 12.00 and 18.50 respectively) from the control at 15 DAT, 25 DAT and 35 DAT. The maximum shoot fresh weight 104.85 g per plant recorded from the treatment T_3 and minimum shoot fresh weight 82.23 g found from the control (T_0) and also maximum shoot dry weight 12.68 g was found from T_3 treatment and minimum 5.95 g was observed from control (T_0). Maximum root fresh weight 12.47 g found from the treatment T_{11} and minimum root fresh weight 8.57 g observed from the control (T_0). On the other hand highest root dry weight 1.19 g found from the treatment T_{11} and lowest weight 0.84 found from control (T_0). Maximum root length 15.37 cm recorded from the treatment T_{11} and minimum root length recorded 11.50 cm from control (T_0). Yield parameters were also significantly influenced by organic and inorganic fertilizers.

Highest antioxidant activities with the half-maximum inhibitory concentration (IC_{50}) was 83.01 µg/ml recorded from the treatment T_3 (Soil 90% + Vermicompost 10%) and lowest value 137.56 µg/ml showed from the treatment T_1. Lower IC_{50} value which means they had significantly higher antioxidant activity. The highest vitamin C value 4.33 mg/100g was found from the treatment T_3 (Soil 90% + Vermicompost 10%) and the lowest value 3.12 mg/100g found from the treatment T_1 (Soil 100% + Inorganic fertilizer). In the studies maximum phenolic contents 87.68 mg GAE/g DW was recorded from the treatment T_3 (Soil 90% + Vermicompost 10%) and minimum phenolic content 53.71 mg GAE/g DW was found from the treatment T_1. Highest flavonoid contents 44.49 mg QE/g DW was recorded from the treatment T_3 (Soil 90% + Vermicompost 10%) and lowest one 26.98 mg QE/g DW found from the treatment T_1.

Vitamin C content in fresh lettuce sample was significantly influenced by different treatments. The lettuce plant under the treatment of cowdung, vermicompost and biochar recorded the maximum value for vitamin C. Organic Lettuce had an excellent performance as regards to phenolic compounds contents and antioxidant potential. The source of organic fertilizer is responsible for the effectiveness in antioxidant efficiency and levels of phenolic compounds. The significantly different TPC and DPPHd scavenging activity between organic and inorganic fertilizers observed in this study suggests that organic fertilizers would be a good choice for the lettuce production.

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APPENDICES

Appendix I. Agra-Ecological Zone of Bangladesh showing the experimental location

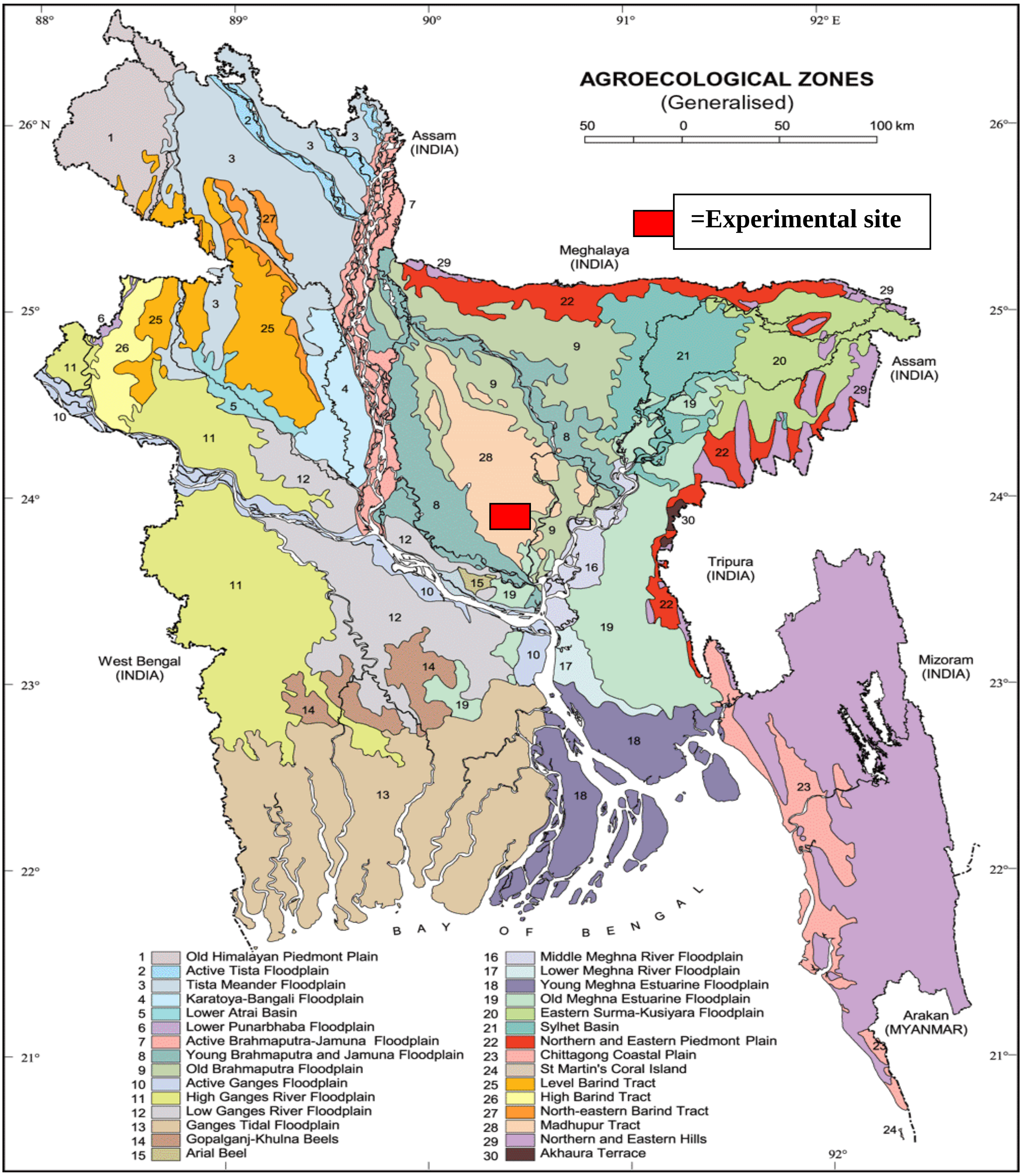

Figure 2: Experimental site

Appendix II: Monthly average air temperature, relative humidity and total rainfall of the experimental site during the period from November 2019 to February 2020.

Year	Month	Air temperature (ºC)			Relative humidity (%)	Rainfall (mm)
		Max	Min	Mean		
2019	November	28.60	8.52	18.56	56.75	14.40
2019	December	25.50	6.70	16.10	54.80	0.0
2020	January	23.80	11.70	17.75	46.20	0.0
2020	February	22.75	14.26	18.51	37.90	0.0

Source: Bangladesh Meteorological Department (Climate division), Agargaon, Dhaka-1212.

Appendix III: Mean square of plant height of lettuce as influenced by organic and inorganic fertilizers.

Sources of variation	Degrees of freedom	Mean square of plant height (cm) at		
		15 DAT	25 DAT	35 DAT
Treatment	11	0.08^{NS}	2.74*	6.48*
Error	36	0.23	0.66	2.94
Total	47			

*significance at 5% level of probability

NS-non significant

Appendix IV: Mean square of number of leaves plant^{-1} of lettuce as influenced by organic and inorganic fertilizers.

Sources of variation	Degrees of freedom	Mean square of number of leaves plant^{-1} at		
		15 DAT	25 DAT	35 DAT
Treatment	11	2.43*	16.29*	13.25*
Error	36	1.01	2.57	2.91
Total	47			

*significance at 5% level of probability

NS-non significant

Appendix V: Mean square of shoots fresh weight and shoots dry weight of lettuce as influenced by organic and inorganic fertilizers.

Sources of variation	Degrees of freedom	Mean square of number of shoot weight at	
		Fresh	Dry
Treatment	11	204.56*	17.85*
Error	36	46.15	1.16
Total	47		

*significance at 5% level of probability

NS-non significant

Appendix VI: Mean square of root length, root fresh weight and root dry weight and of lettuce plant as influenced by organic and inorganic fertilizers.

Sources of variation	Degrees of freedom	Mean square of root at		
		Length	Fresh weight	Dry weight
Treatment	11	6.37*	5.39*	0.04*
Error	36	0.92	1.21	0.01
Total	47			

*significance at 5% level of probability

NS-non significant

Appendix VII: Mean square of vitamin C of lettuce leaf as influenced by organic and inorganic fertilizers.

Source of variation	Degrees of freedom	Mean square
		Vitamin C of lettuce leaf
Treatment	11	0.566*
Error	36	0.148
Total	47	

*significance at 5% level of probability

NS-non significant

Appendix VIII: Mean square of Antioxidant activity of lettuce leaf as influenced by organic and inorganic fertilizers.

Source of variation	Degrees of freedom	Mean square
		IC_{50} Value of lettuce leaf
Fertilize	11	921.403*
Error	36	104.690
Total	47	

*significance at 5% level of probability

NS-non significant

Appendix IX: Mean square of Total phenolic content of lettuce leaf as influenced by organic and inorganic fertilizers.

Source of variation	Degrees of freedom	Mean square
		Total phenolic content of lettuce leaf
Fertilize	11	528.86*
Error	36	51.52*
Total	47	

*significance at 5% level of probability

NS-non significant

Appendix X: Mean square of total flavonoid content of lettuce leaf as influenced by organic and inorganic fertilizers.

Source of variation	Degrees of freedom	Mean square
		Total flavonoid content of lettuce leaf
Fertilize	11	156.799*
Error	36	11.557*
Total	47	

*significance at 5% level of probability

NS-non significant