Growth and production of several Romaine lettuce varieties 
(*Lactuca sativa* var. Romana) on various ratios of ammonium-nitrate in hydroponic nutrition formulations

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Abstract. The study aimed to determine the response of four varieties of romaine lettuce, grown hydroponically, to Ammonium-Nitrate ratio in the hydroponic solution as a source of N nutrient. The research was conducted in a Screen House at Tamalanrea district of Makassar from October to December 2018 in the form of factorial experiments based on nested design patterns. The trial used three ratios of Ammonium-Nitrate consisted of 1:4, 1:6, and 1:8 into Hougland hydroponic nutrient solution, and AB Mix as the control treatment. Four Romaine Lettuce varieties used as the second factor consisted of Romaine Ballon, Swiss Jade, Red Romaine, and Green Romaine. The results show that the ratio of Ammonium-Nitrate of 1:4 gave the best growth and production while Romaine Ballon variety showed better growth and production compared to other varieties.

1. Introduction

Lettuce is one of the vegetables that have a promising market prospect. This vegetable has a high nutritional content and is very popular in the community. The demand for lettuce has not been maximally fulfilled, and this is because there are obstacles in its cultivation. One way to produce high-quality vegetable products continuously with high quantity is to cultivate it in a hydroponic system [1].

Hydroponics is a system of crop cultivation without using soil as a medium for growing plants with additional nutrients for growth. The advantage of cultivating with a hydroponic system is that the hygiene of plants is easier to maintain, no land management and weed control are needed, sterile planting media, an efficient use of water and fertilizer, off-seasonal plants cultivation, can be done on a narrow, protected area from rain and direct sun [2].

The success of hydroponic systems is influenced by several factors, and one of them is the availability of nutrients. The availability of quality hydroponic nutrients plays an important role in the success of the production of commodities that are cultivated hydroponically [3]. Addition of nutrients is necessary for the cultivation of plants with hydroponic systems, both essential nutrients of macro and micro. The nutrient solution provided consists of macro and micro salts made in stock solutions A and B in AB Mix [4].

Leafy vegetables need a fertilizer with sufficient nitrogen element for the vegetables to grow well, crunchier, fresh, and edible [5]. This finding is in line with the results of Mahanani [6] research on Pak Choy leaf vegetables which found that the use of N nutrients in Pak Choy plants increased leaves...
chlorophyll which are used for the formation of amino acids and proteins. While in control plants resulted in smaller leaf size and earlier leaf senescence because the N nutrients available was not enough to form protein and chlorophyll, causing declined ability of the plant to perform important metabolism in carbohydrate synthesis.

Nitrogen is generally absorbed by plants in the form of $\text{NH}_4^+$ or $\text{NO}_3^-$, which is influenced by the nature of the soil, types of plants, and stages in plant growth [7]. According to Muharja [8], in formulating the hydroponic fertilizer for leafy vegetables, the ratio of ammonium-nitrate ($\text{NH}_4^+:\text{NO}_3^-$) is six, meaning it would need six nitrates and one ammonium, while the total N is 250 ppm. Based on this background, this study aims to determine the ratio of ammonium and nitrate which gives the best influence on the growth and production of romaine lettuce plants and to determine the romaine lettuce varieties that have the best growth and production.

2. Methodology
The research was carried out in a controlled environment condition in a Screen House located in Tamalanrea District, Makassar City from October to December 2018. This study used a factorial experimental design in a nested pattern with the factor of varieties nested in nutritional factors. Nutritional factors consisted of four levels, namely ABMix as control (N1), modification of the hydroponic nutrient formulation from Hougland solution consisting of the ratio of Ammonium-Nitrate of 1:4 (N2), the ratio of Ammonium-Nitrate of 1:6 (N3), and the ratio of Ammonium-Nitrate of 1:8 (N4), respectively. The varieties tested consisted of four Romaine lettuce varieties, namely Romaine Ballon (V1), Swiss Jade (V2), Red Romaine (V3), and Green Romaine (V4). The data obtained were then analyzed using analysis of variance and if the results were significant, then continued with the LSD test at the level of 5%. The observation parameters consisted of fresh weight of the root and canopy, number of leaves, leaf area, stomatal density, chlorophyll a, chlorophyll b, total chlorophyll, and production.

3. Results
The experimental results show that the formulation of nutrition had a significant effect on the fresh root weight, shoot fresh weight, the number of leaves, and economic yield of the romaine lettuce (figure 1). Similarly, the use of different ratios of Ammonium-Nitrate in the modified Hougland hydroponic solution significantly affected leaf area, stomatal density, and the level of chlorophyll a, b and total chlorophyll content of the leaves (figure 2).

![Figure 1](image1.png)

Figure 1. Graph of the effect of nutrient formula on fresh root weight, shoots, number of leaves, and economic yield of Romaine lettuce Numbers followed by the same letters on each parameter are not significantly different (LSD $\alpha$ 0.05).
Leaf area (cm²)  Stomatal density (n.cm⁻²)  Chlorophyll a (µ.m⁻²)  Chlorophyll b (µ.m⁻²)  Total chlorophyll (µ.m⁻²)

Figure 2. Graph of the effect of nutrient formula on leaf area, stomatal density, chlorophyll a, b, and total chlorophyll (B). Numbers followed by the same letters on each parameter are not significantly different (LSD α 0.05).

Figure 3. The effect of varieties on fresh weight of roots, shoots, number of leaves, and economic yield of the Romaine lettuce plants. Numbers followed by the same letters on each parameter are not significantly different (LSD α 0.05).

The ammonium-nitrate ratio of 1:4 in the modified composition of Hougland solution resulted in higher fresh weight of roots, shoots, leaf number and economic production compared to the other treatments of AB mix solution, Ammonium, and Nitrate ratio of 1:6 and 1:8 (figure 1). The Ammonium and Nitrate ratio of 1:4 in the modified Hougland nutritional solution resulted in better development of leaf area and higher stomatal density, the content of chlorophyll a, b, and total chlorophyll compared to other nutritional formulation treatments (figure 2). The Romaine varieties differed significantly in the fresh weight of roots, shoots, number of leaves, and economic yield. Variety of Romaine Ballon showed better growth and production compared to other varieties (figure 3).

4. Discussion
Nitrogen is an important macronutrient needed by plants in the highest amount compared to all other mineral elements. Generally, N is supplied to plants in the form of nitrate (NO₃⁻), ammonium (NH₄⁺),
or urea [CO(NH$_2$)$_3$] [9]. The level and form of N fertilizer can affect plant growth and must be managed appropriately to maximize plant growth and development.

The results in this study show that the use of ammonium and nitrate in the ratio of 1:4 in the hydroponic nutrient solution resulted in better growth and development of lettuce plant. The effects are shown from a higher vegetative component such as root and canopy fresh weight, better leaf area, hence increased economic yield. The same ratio formula of ammonium and nitrate also increased leaf physiological characters, namely stomatal density, the content of chlorophyll a, b and total chlorophyll compared to the use of AB mix and the other NH$_4^+$ and NO$_3^-$ ratio (1:6 and 1:8). The effect was not significantly different in all varieties used; however, the Romaine Balkon variety was shown to have the highest response to the ratio of NH$_4^+$/NO$_3^-$ used in the trial.

The results obtained were inversely proportional to the results of Wang and Shen [10] which reported that the use of NH$_4^+$/NO$_3^-$ the ratio of 25:75 in hydroponic nutrition on five lettuce genotypes showed better root and shoot growth compared to lower NH$_4^+$/NO$_3^-$ ratio. The difference in the results obtained is probably caused by the lettuce genotype used. In the previous study [10], Sx 1 genotype showed the most sensitive response to changes in the ratio of NH$_4^+$/NO$_3^-$ while the Nmct genotype was less responsive to the source ratio of NH$_4^+$/NO$_3^-$ as a source of nitrogen in lettuce plant hydroponic cultivation.

The threshold solution for the concentration of NH$_4^+$ in the hydroponic nutrient solution of the lettuce is 9-12% of the total N in the nutrient solution [11]. Increasing the concentration of NH$_4^+$ in the hydroponic solution of lettuce plants resulted in a decrease in the value of the observed parameters which may be caused by concentrations of NH$_4^+$ that are too high in the nutrient solution which will resulted in the declined absorption rate of cations especially K$^+$ as a consequence of ion imbalance [12]. Potassium in plants is involved in photosynthetic activity through its role in stimulating the process of opening and closing the stomata. The opening of the stomata is caused by a large number of K$^+$ ions contained in the guard cell so that it can result in a reduction in osmotic potential and followed by increased cell turgor pressure [9].

Root growth is reduced at higher concentrations of NH$_4^+$ in the nutrient solutions. This observation might not be attributed to the direct effects of NH$_4^+$ toxicity. Root growth has been considered as the dependence of carbon/nitrogen balance between shoot and roots [13]. The current hypothesis underlying these models has considered that roots grow to look for water and nutrients, especially nitrogen.

One of the questions that emerged from this experiment was the negative effect of NH$_4^+$ concentration on the growth and development of lettuce plants. Both of these plant's physiological processes can be influenced by salinity or ion toxicity. In a previous paper [11], it has been shown that only fresh weight is reduced by the salinity of nutrient solutions in the range of concentrations between 1.0 and 5.0 dSm$^{-1}$. This reduction is related to restrictions in the water flow of plants due to the effect of salinity. Other physiological processes appear to be involved in NH$_4^+$. Increasing the concentration of NH$_4^+$ will result in lower Mg uptake levels [9].

Consequently, the synthesis of leaves chlorophyll a, b and total chlorophyll were retarded and will ultimately reduce the rate of photosynthesis, hence a lower weight of roots, leaves, and leaf area formed. Decreasing Mg uptake is the consequence of the antagonistic properties of the N-ammonium form with Mg uptake in nutrient solutions [14]. In Pisum sativum L., chlorophyll biosynthesis begins with the insertion of Mg into protoporphyrin, which is catalyzed by Mg$^{2+}$-chelatase [15].

5. Conclusion

Based on the results of the research, it can be concluded that:

- The ratio of ammonium-nitrate 1:4 in hydroponic nutrient solutions of hydroponically grown lettuce resulted in the best growth and production with an economic yield of 182.71 g per plant.
- Romaine Ballon variety showed the best growth and production with an economic yield of 185.83 g per plant.
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