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Original Research Article

# Comparing Sources of Nitrogen Fertilizer on Growth in Sunflower Microgreens

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**ABSTRACT**

Sunflower microgreens are an important protein source and essential amino acids, but the production of microgreens still has a very low potential. Because of the nutrients that the plant needs are not added to the production of microgreens. In this study, sunflowers were grown with coconut coir and fertilizers were sprayed with seven different nitrogen nutrient solutions (monoammonium phosphate, potassium nitrate, calcium nitrate, ammonium sulfate, ammonium nitrate, urea, and monosodium glutamate). Fresh and dry weights, chlorophyll a, chlorophyll b, carotenoid, and xanthophyll were assessed and microgreens were grown for seven days. The results showed that the sunflower microgreens sprayed with deionized water showed fresh weight, dry weight and contents of chlorophyll a, chlorophyll b, carotenoids and xanthophyll not different with other nitrogen source treatments. Therefore, the result of this study indicates that nitrogen sources (26.5 mM N) did not affect fresh weight, dry weight and the contents of chlorophyll a, chlorophyll b, carotenoids and xanthophyll in sunflower microgreens.



## INTRODUCTION

Currently, the world population is an aging society and working age. People pay more attention to food choices and choose healthy foods such as fruits and vegetables, which are safe and contain essential nutrients for their healthy. In addition, vegetables are very important to human health as they contain vitamins, minerals, chemical compounds and dietary fibres (Slavin and Lloyd, 2012). Suitable consumption of vegetables can prevent certain chronic diseases such as diabetes, cancer, obesity, metabolic diseases, cardiovascular disease and improve risk factors associated with these diseases (Ülger et al., 2018). Many types of vegetables are popular among health lovers, one among them is the microgreen vegetables (Kozai et al., 2020). But current microgreen production still has low potential. Because it does not add nutrients needed for plant growth such as nitrogen (N), which nitrogen is an

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essential element for all living things. Although the earth's atmosphere contains 78% nitrogen, some forms of usable nitrogen are limited for biological applications, especially plant (Htwe and Ruangrak, 2020; Vitousek and Howarth, 1991). Nitrogen is a vital element for plant growth, development and reproduction (Leghari et al., 2016). Moreover, nitrogen is also the main component of chlorophyll, a compound that plants gather energy from the sunlight, water and carbon dioxide to produce the sugar or it is called photosynthesis system (Bassi et al., 2018). Significantly, nitrogen is the key of producing amino acids and the structure blocks of proteins. The proteins are important for plant which can be shriveled and died without proteins (Masclaux-Daubresse et al., 2010). Although, nitrogen is one of the richest elements on the earth, but nitrogen deficiency is still common problem in crop production around the world. Because of nitrogen form from the atmosphere cannot be used directly by any crop (Anas et al., 2020). However, nitrogen deficiency in crops affects to decrease photosynthetic ability and efficiency of carboxylation because the structural compounds in photosynthesis such as chlorophyll and ribulose bisphosphate carboxylase (Rubisko) are reduced and related to decrease production of glutamine and a precursor to many amino acids (Bagh et al., 2004; Delgado et al., 1994). In crop production, nitrogen element is one of the major costs for many crops, except for legume crops that can directly absorb nitrogen from the atmosphere by nitrogen fixing bacteria. In every year, nitrogen fertilizers have been used approximately 85–90 million metric tons (MMt) which are applied to cultivation, worldwide and it is predictable to increase more than 240 million tons by 2050 (Good et al., 2004).

The nitrogen transportation in the plant has many steps for example nitrogen absorption, assimilation, and translocation (Lea and Miflin, 1974; Malagoli et al., 2005). Moreover, the nitrogen in old leaves can be reused or recycled and remobilized to young leaves (Htwe and Ruangrak, 2020; Malagoli et al., 2005). Plant up takes the nitrogen molecules in form of inorganic including nitrates and ammonium. It also can up take nitrogen molecules in form of organic including amino acids. Uptake of inorganic nitrogen is required to reduce nitrate to nitrite and ammonium. Nitrate is one of the nitrogen molecules forms and nitrogen fertilizers used for plant growth and development. Nitrates are used as a precursor of protein production in the plant. Plants absorb nitrates via roots up to the shoots to reduce and maintain in the vacuoles (Tischner, 2000). The reduction of nitrate to nitrite occurs in the cytosol by nitrate reductase. In addition, nitrate reduction occurs both in roots and shoots. In the chloroplasts, nitrite is reduced to ammonium by nitrite reductase after nitrate is reduced to nitrite and nitrite is transported to chloroplasts, where it is formed to be ammonium by the nitrite reductase (Lea and Forde, 1994; Lea and Miflin, 1974), then converted to glutamine and glutamate acid by the enzyme glutamine synthase and glutamate dehydrogenase, respectively. Finally, it is combined to be an amino acid, proteins and other organic nitrogen compounds (Meyer and Stitt, 2001).

Nitrate is important nitrogen source that is used in agricultural practice and it affects the circadian rhythms, reduces carbon and increases nitrogen requirements (Crawford and Forde, 2002). Lack of nitrate affects to decrease chlorophyll content and turn leaf to be pale green or yellow. It directly affects to reduce the photosynthetic ability, growth and crop yield, thereby reducing farmers' income. Consequently, there should increase needed levels of nitrate concentration in the soil by applying sources of nitrate in forms of the chemical or natural fertilizers (Hasnain et al., 2020). In addition, ammonium is one of nitrogen form used in agricultural sector for plant growth and development. Usually, plants need to reduce nitrate to ammonium and then assimilated into organic compounds (Bloom et al., 1992). Typically, ammonium is absorbed faster than nitrate by plant (Clarkson et al., 1986; Fried et al., 1965; Macduff and Jackson, 1991). It is also reported that urea is the most widely used as a nitrogen fertilizer in agriculture on a global scale (Stalin and Sukumaran, 2021). In plant production, urea has been used about half of the total nitrogen. Urea enters to the plants either directly or in the forms of ammonium or nitrate after urea is degraded by soil microorganisms (Witte, 2011). It plays a role as the primary nitrogen source and an intermediary of plant catalysis of arginine. Urea exerts pressure to suppress the influx of nitrates, while urea increases the absorption of ammonium. Urea bioavailability is less effective than nitrate absorption and plants grown with urea have signs of nitrogen deficiency (Mérigout et al., 2008). In addition, the first organic nitrogen compounds are glutamate and glutamine. They obtain from the uptake of nitrates and ammonium in plants. The major pathway of nitrogen absorption, nitrate is extracted from the soil which it is reduced to nitrite and ammonium by nitrate and nitrite reductase. Ammonium obtains from nitrates or directly absorbs from the soil. It can be changed into glutamine and glutamate through the glutamine synthase and glutamine-oxoglutarate aminotransferase cycle (Lam et al., 1995; Lea and Miflin, 1974; Tabuchi et al., 2007). On the other hand, plant can get glutamine and glutamate from biodegradable of other amino acids such as ornithine, proline, and arginine (Forde and Lea, 2007; Kan et al., 2017). In chloroplasts, the glutamine synthase, glutamine-α-oxoglutarate transaminase cycle and glutamate dehydrogenase can synthesize the glutamate. It is same as proline/pyroline 5-carboxylate cycles in plant cytoplasm. In addition, glutamate can be transported from mitochondria and chloroplast to cytoplasm through glutamate transporters. Then, glutamate decarboxylase catabolizes glutamate to γ-aminobutyric acid. Afterward, γ-aminobutyric acid is carried and changed by γ-aminobutyric acid transaminase to be succinic semaldehyde and leads into mitochondria. Succinate is then produced by catalyzing the succinate semialdehyde dehydrogenase reaction in cycle of the tricarboxylic acid. The TCA cycle produces 2-oxoglutarate or α-ketoglutarate and then converts to glutamate by glutamate dehydrogenase (Qiu et al., 2020). After that, the glutamate from mitochondria can be transported into the cytoplasm by glutamate transporters. However, it can be degraded to be γ-aminobutyric acid (Robinson et al., 2020).

The nitrate, ammonium, urea and monosodium glutamate are used as nitrogen sources for promoting plant growth, yield and quality around the world. Therefore, this study focused on comparing the sources of nitrogen fertilizer on growth in the sunflower microgreens to be an ideal source of nitrogen fertilizer that can increase the growth and quality of sunflower microgreens for making healthy food choice.

## MATERIALS AND METHODS

### Experimental design and treatment

This study used a Randomized Completely Block Design (RCBD) for the experimental design. There were eight treatments including (1) monoammonium phosphate ($NH_4H_2PO_4$), (2) ammonium sulphate ($(NH_4)_2SO_4$), (3) ammonium nitrate ($NH_4NO_3$), (4) potassium nitrate ($KNO_3$), (5) calcium nitrate ($Ca(NO_3)_2$), (6) Urea ($CH_4N_2O$), (7) monosodium glutamate ($C_5H_8NaO_4$) and (8) Deionized (DI) water (control treatment). Seven treatments of nitrogen fertilizer were set up with the same total nitrogen contents. These treatments were applied at 26.5 mM N. The experiment was repeated five times, then 40 experimental units were obtained. Microgreens harvested at seven days after seedling.

**Plant materials and growth conditions**

The experiment was implemented in 2021 at laboratory of Urban Agriculture Technology, Department of Agricultural and Fishery Science, Faculty of Science and Technology, Prince of Songkla University, Pattani Campus (26º 35´ N, 106º 420´ E), Thailand. The sunflower seeds were used and eight clean empty trays (60×25 cm) were used. After that, coconut coir was applied to be as a growing medium and placed on each tray and watered with distilled water. The sunflower seeds needed to soak in water at the room temperature and then, they were sown on the coconut coir layers of each tray and they were covered up with a layer of coconut coir again. 100 grams of sunflower seeds were prepared eight trays. Empty trays were covered on the top of the sowed seed tray for two days. After that, the trays were opened and sprayed 1000 mL of different fertilizer solutions as followed each treatment. Then, they were Harvested 7 days after sowing (2 days after treatment). and washed with distilled water. Finally, they were kept in a zip lock bag under 4 Cº.

**Table 1.** The different types of nitrogen fertilizers

| | Treatment | mg/L |
|---|---|---|
| 1 | Monoammonium Phosphate ($NH_4H_2PO_4$) | 217.393 |
| 2 | Ammonium Sulphate ($(NH_4)_2SO_4$) | 249.618 |
| 3 | Ammonium Nitrate ($NH_4NO_3$) | 151.277 |
| 4 | Potassium Nitrate ($KNO_3$) | 191.078 |
| 5 | Calcium Nitrate ($Ca(NO_3)_2$) | 310.122 |
| 6 | Urea Fertilizer ($CH_4N_2O$) | 113.502 |
| 7 | Monosodium Glutamate ($C_5H_8NNaO_4$) | 319.594 |
| 8 | Deionized Water (control) | 0 |

**Measurements of fresh weight and dry weight**

For fresh weight, microgreens were gently blotted with soft tissue paper to remove any free surface moisture. They were weight immediately. For dry weight, microgreens were dry in hot air oven that was set to 65°C for 72 hours.

**Determination of chlorophyll a, chlorophyll b, carotenoids and xanthophyll**

The chlorophylls, carotenoids and xanthophylls were extracted with ethanol according to the modified methods as described by Duma et al. (2014).

Extraction of pigments from seedlings. 2 g of fresh weight in sunflower microgreen were ground in a mortar with 5 ml of ethanol 95% grinding continued until homogeneous state, then filtered through with a filter paper (no. 1). 250 µL of sample and blank into a 96-well. The absorption measurements (A) at 440, 480, 495, 649, and 665 nm with a Microplate Spectrophotometer (EZ Read 2000 Microplate Reader) and calculated according to the equation.

$$\text{Chlorophyll a (mg g}^{-1}) = \frac{\mathbf{13.7}A665 - \mathbf{5.76}A649}{mass \times 200}$$

$$\text{Chlorophyll b (mg g}^{-1}) = \frac{\mathbf{25.8}A649 - \mathbf{7.6}A665}{mass \times 200}$$

$$\text{carotenoids (mg g}^{-1}) = \frac{\mathbf{4.7}A440 - \mathbf{0.263}chl\ a + chl\ b}{mass \times 200}$$

$$\text{xanthophyll (mg g}^{-1}) = \frac{11.51A480 - 20.61A495}{\text{mass} \times 200}$$

**Statistical analysis**

The data were analyzed by the analysis of variance (ANOVA), if there were significant differences between treatments, further tests were carried out using the Duncan's at a 95% ($p < 0.05$) level. Statistical analysis was performed using SPSS 24.0 for Windows.

## RESULTS AND DISCUSSION

**Fresh weight and dry weight**

The results of fresh weight and dry weight of sunflower microgreens showed that effect of different nitrogen sources on sunflower microgreens were not significantly different as show as in Table 2. However, Murphy et al. (2010) have found that arugula microgreens were influenced in the order of calcium nitrate, ammonium nitrate and urea, respectively. In addition, fresh weight shoot/square meter or square meter was also influenced by calcium nitrate, ammonium nitrate, and urea, these three nitrogen sources resulted in fresh weight/square meter more than ammonium sulfate (Murphy and Pill, 2010).

**Determination of chlorophyll a, chlorophyll b, carotenoid and xanthophyll**

The results of chlorophyll a contents showed highest in sunflower microgreens sprayed with ammonium nitrate (0.047 mg/g), following Urea (0.046 mg/g), ammonium sulphate (0.045 mg/g), deionized water (0.045 mg/g), calcium nitrate (0.044 mg/g), potassium nitrate (0.041 mg/g), monosodium glutamate (0.040 mg/g) and monoammonium phosphate (0.036 mg/g) (Figure 1). However, chlorophyll a content of sunflower microgreens sprayed with ammonium nitrate was not significant difference with treatments of calcium nitrate, ammonium sulphate, urea and deionized water. Moreover, sunflower microgreens under treatments of monoammonium phosphate, potassium nitrate, calcium nitrate, ammonium sulphate, urea, monosodium glutamate and deionized water showed that chlorophyll a contents were not significant difference (Figure 1).

**Table 2.** Effects of monoammonium phosphate, potassium nitrate, calcium nitrate, ammonium sulphate, ammonium nitrate, urea fertilizer, monosodium glutamate and deionized water (control) on fresh weight and dry weight of sunflower microgreens

| Plant | Treatment | Fresh weight (g/100 seedling) | Dry weight (g/100 seedling) |
|---|---|---|---|
| Sunflower Microgreen | Monoammonium Phosphate | 41.18 ± 7.16 | 2.69 ± 0.29 |
| | Potassium Nitrate | 40.70 ± 7.43 | 2.65 ± 0.26 |
| | Calcium Nitrate | 39.14 ± 5.53 | 2.65 ± 0.27 |
| | Ammonium Sulphate | 40.30 ± 7.89 | 2.70 ± 0.29 |
| | Ammonium Nitrate | 39.80 ± 6.60 | 2.72 ± 0.28 |
| | Urea Fertilizer | 41.30 ± 7.75 | 2.73 ± 0.31 |
| | Monosodium Glutamate | 40.63 ± 9.01 | 2.62 ± 0.37 |
| | Deionized Water (control) | 38.79 ± 7.36 | 2.65 ± 0.27 |

Results are means ± Standard Deviation. Means not significantly different at $p < 0.05$ according to Duncan's test.

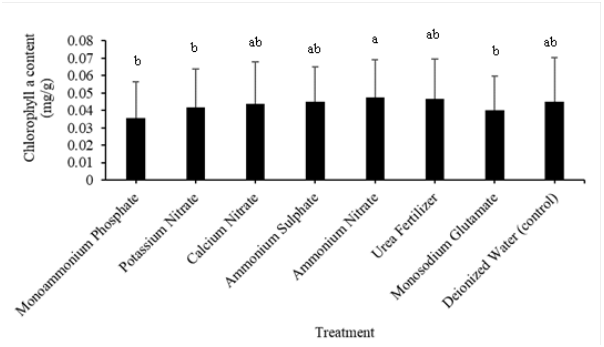


**Figure 1.** Effects of monoammonium phosphate, potassium nitrate, calcium nitrate, ammonium sulphate, ammonium nitrate, urea fertilizer, monosodium glutamate and deionized water (control) on chlorophyll a content (mg/g) in sunflower microgreen. Different letters indicate significant differences ($p < 0.05$) according to Duncan's test.

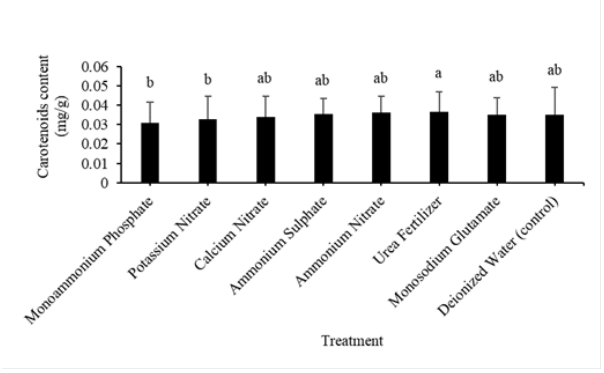


**Figure 2.** Effects of monoammonium phosphate, potassium nitrate, calcium nitrate, ammonium sulphate, ammonium nitrate, urea fertilizer, monosodium glutamate and deionized water (control) on chlorophyll b content (mg/g) in sunflower microgreen. Different letters indicate significant differences ($p < 0.05$) according to Duncan's test.

Sunflower microgreens were sprayed with ammonium nitrate (0.032 mg/g), the chlorophyll b contents showed highest and followed by Urea (0.030 mg/g), ammonium sulphate (0.029 mg/g), calcium nitrate (0.029 mg/g), monosodium glutamate (0.028 mg/g), deionized water (0.028 mg/g), potassium nitrate (0.027 mg/g) and monoammonium phosphate (0.026 mg/g) (Figure 2). However, treatment of ammonium nitrate showed the result of the chlorophyll b contents was not significantly different with ammonium sulphate and urea treatments. Furthermore, the sunflower microgreens grown under treatments of monoammonium phosphate, potassium nitrate, calcium nitrate, ammonium sulphate, urea, monoammonium phosphate and deionized water showed the chlorophyll b contents not significantly different (Figure 2).

The carotenoids content in sunflower microgreens grown under treatment of urea (0.0365 mg/g) showed highest, following ammonium nitrate (0.0362 mg/g), ammonium sulphate (0.0355 mg/g), monosodium glutamate (0.0350 mg/g), deionized water (0.0350 mg/g), calcium nitrate (0.0338 mg/g), potassium nitrate (0.0327 mg/g), and monoammonium phosphate (0.0307 mg/g) (Figure 3). However, urea treatment did not different with treatments of calcium nitrate, ammonium sulphate, ammonium nitrate, monosodium glutamate and deionized water. Moreover, the carotenoids content grown under treatment of ammonium nitrate, ammonium sulphate, monosodium glutamate, deionized water, calcium nitrate, potassium nitrate, and monoammonium phosphate were not significant difference (Figure 3).

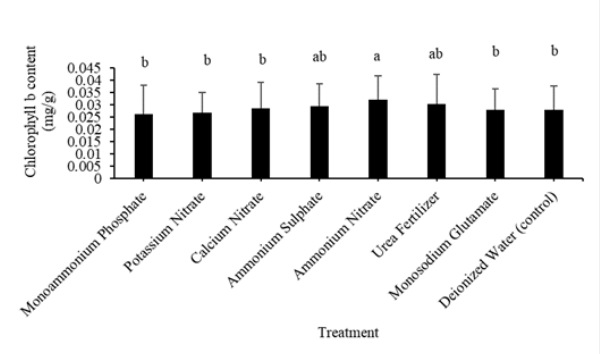


**Figure 3.** Effects of monoammonium phosphate, potassium nitrate, calcium nitrate, ammonium sulphate, ammonium nitrate, urea fertilizer, monosodium glutamate and deionized water (control) on carotenoid content (mg/g) in sunflower microgreen. Different letters indicate significant differences ($p < 0.05$) according to Duncan's test.

The xanthophyll contents were show highest in sunflower microgreens under urea treatment (0.0214 mg/g) and followed by

ammonium nitrate (0.0208 mg/g), monosodium glutamate (0.0204 mg/g), deionized water (0.0197 mg/g), calcium nitrate (0.0183 mg/g), ammonium sulphate (0.0180 mg/g), potassium nitrate (0.0179 mg/g) and monoammonium phosphate (0.0164 mg/g) (Figure 4). However, urea treatment was not significantly different with ammonium nitrate, monosodium glutamate and deionized water. There were also were not significant between treatments of monoammonium phosphate, potassium nitrate, calcium nitrate, ammonium sulphate, ammonium nitrate, monosodium glutamate and deionized water (Figure 4).

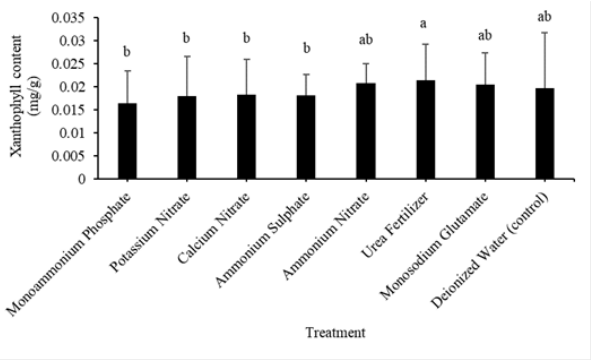


**Figure 4** Effects of monoammonium phosphate, potassium nitrate, calcium nitrate, ammonium sulphate, ammonium nitrate, urea fertilizer, monosodium glutamate and deionized water (control) on xanthophyll content (mg/g) of sunflower microgreen. Different letters indicate significant differences ($p < 0.05$) according to Duncan's test.

Chlorophyll and carotenoids are major photosynthetic pigments responsible for the specific coloration of microgreens (Kowitcharoen et al., 2021; Žnidarčič et al., 2011). When decreasingnitrogen concentration consequences chlorophylls, β-carotene, neoxanthin, lactucax-anthin, and all trans- and cis-violaxanthin decreased (Becker et al., 2015). Nitrate nitrogen plays important role in the transcription and posttranscriptional regulation of nitrogen assimilation and chlorophyll synthesis pathway enzymes (Wen et al., 2019). Nitrate and ammonium are the main forms of nitrogen for plants. Excessive ammonium accumulation in tissues is toxic for plants. But arabidopsis thaliana natural accessions grown under 1mM ammonium or 1 mM Nitrate. chlorophyll content increased in every accession upon ammonium nutrition. While mild ammonium stress induces chlorophyll accumulation (Sanchez Zabala et al., 2015). exogenous ammonium concentration and the increased accumulation of ammonium in the roots, resulting in photosynthetic activity, total Chlorophyll and the concentration of carbohydrates increased. In addition, the concentration of root protein was increased. showed that with the incorporation of ammonium into organic nitrogen The activity of glutamine synthase (GS) in roots was increased by ammonium below 5 mM, whereas the activity of glutamate dehydrogenase was increased by ammonium. (GDH) in the roots increased with the amount of ammonium (Horchani et al., 2010). It is also reported that ammonium concentration ranged from 2 to 10 mM. The protein and chlorophyll content increased. And it can be said that ammonium is a factor that regulates the formation and function of protein synthesis (Smolov and Semenova, 2008). But plant growth responses show that plants prefer ammonium over nitrate as an inorganic nitrogen source. When given nitrates alone Plants will show signs of nitrogen deficiency. Shoot thickness and chlorophyll reduce the nitrogen concentration of young leaves (Garbin and Dillenburg, 2008). Nitrogen deficiency increases the concentration of flavonoid glycosides and caffeic acid derivatives. This resulted in lower concentrations of chlorophyll a and b, beta-carotene and xanthophyll (Becker et al., 2015). In addition, urea is a more appropriate substrate to produce chlorophyll and carotenoids (Kumawat et al., 2015).

Microgreens had chlorophylls and carotenoids than sprouts (Wojdyło et al., 2020). Carotenoids play an important role in the protection of plants against photooxidative processes (Alrifai et al., 2019). They are also efficient antioxidants (Mir et al., 2017; Weber, 2017). Consumption of carotenoid-rich products has been demonstrated to bring health benefits in the form of ameliorating degenerative and cardiovascular diseases and in the protection of humans against cancers of, e.g., liver, colon, lung, pancreas or prostate (Choe et al., 2018; Mir et al., 2017; Treadwell et al., 2020; Wojdyło et al., 2018).

## CONCLUSIONS

Our studies aim to compare the sources of nitrogen fertilizer on growth in the sunflower microgreens to be a source of nitrogen fertilizer that can increase the growth of sunflower microgreens and make microgreens a quality healthy food choice. Nitrogen fertilizers were set up with the same total nitrogen contents. These treatments were applied at 26.5 mM N. Showed that ammonium nitrate (151.277 mg/L) can increase chlorophyll a and chlorophyll b. In addition, the use of urea (113.502 mg/L) can increase carotenoid and xanthophyll. They affect plant growth and these plants beneficial to human health.

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