

Use of Seaweed Liquid Extract and Cow Urine for Lettuce Growth in Hydroponic System

Laode Muhammad Harjoni Kilowasid^{1,*}, Sitti Fadhillah Arsani¹, Tresjia Corina Rakian¹, Rachmawati Hasid¹, Waode Siti Anima Hisein², and Andi Nurmas¹

¹ Department of Agrotechnology, Agriculture Faculty, Halu Oleo University, 93232, Kendari, Indonesia

² Department of Plant Protection, Agriculture Faculty, Halu Oleo University, 93232, Kendari, Indonesia

Abstract. Lettuce cultivated by millennial farmers using a hydroponic system in urban areas. Seaweed extracts and cow urine contain nutrients and plant growth regulators. This study aims to determine the effect of a formulation containing inorganic nutrient solution, seaweed liquid extract, and mixed cow urine on lettuce growth, as well as the volume of commercial nutrient mixture that can be substituted by these additives in a hydroponic system. The treatment used contains a combination of inorganic fertilizer solution (CF), seaweed liquid extract (SLE), and cow urine (CU). Every repeated three times in a randomized block design. Results, the nutrient mixture had a significant effect on the height, leaves number as well as wet and dry weight of the plants. Lettuce planted on 50% CF+ 25% SLE+ 25% CU was the tallest when observed on days 7, 14, and 42 after planting. The highest fresh and dry weight was also obtained from this nutrient formulation. On day 35, the highest leaves number was recorded from plants on the 100% CF+ 0% SLE+0% CU medium. Concluded, a mixture of seaweed liquid extract and cow urine has the potential to reduce the inorganic fertilizers use as a nutrient solution in a hydroponic system.

1 Introduction

The consumption of a functional food diet helps to meet nutritional needs, prevent mineral deficiency, as well as support the growth and maintenance of the body [1] due to their vitamins, minerals, and antioxidants content. Food that contains a sufficient amount of these nutrient are obtained from leafy vegetables, such as lettuce (*Lactuca sativa* L), which is often consumed in the form of fresh, soup or mixed salad. Furthermore, the leaves contain sugar, protein, and minerals, such as Ca, Mg, Na, K, Fe, Zn, and Mn as well as antioxidants, including carotenoids, flavonoids, -carotene, chlorophylls, phenolics, anthocyanins, and ascorbic acid. These compounds help to improve the nutritional intake in the diet [2,3]. Lettuce leaves also contain nutrients that are low in calories, and this is very beneficial to health because it reduces the risk of cardiovascular disease, diabetes, and colon cancer [4].

* Corresponding author: lohadjoni2@yahoo.co.id

The number of people in urban areas is expected to continue increasing up to 68% of the total world population [5]. Furthermore, the consumption of lettuce in these areas is also expected to increase due to the development of various culinary delights produced from processed lettuce leaves. This increased rate has triggered creative and innovative public interest, both individually and in groups, to become millennial farmers because the cultivation business is an economic opportunity to increase their welfare. Lettuce is often cultivated using a hydroponic system, where the plants are placed in a mineral nutrient solution, such as AB mix using water as a solvent without soil [6]. However, their relatively high price is a limiting factor for novice millennial farmers with financial limitations to produce lettuce with this technique. The complete use of inorganic fertilizers as a source of mineral nutrients also has negative effects on the environment, which is a major concern, especially in urban areas. [7]. In line with the evolution of agricultural production, the approach in agronomic practices has shifted from the intensification of the green revolution era to that of sustainable agriculture [8]. The new approach entails making efforts to efficiently use inorganic fertilizers as well as protect the environment by reducing pollution without affecting crop production [9]. This evolution has also influenced soilless plant cultivation systems, especially the hydroponic method [10]. Therefore, the system needs various nutrient sources that are relatively cheaper, easy to obtain, and help to overcome these environmental risks.

Urine excreted by cows contains more than 90% N-urea [11], hence, it can become the major source of ammonia emission (NH₃) in the atmosphere as well as a eutrophication agent in water bodies when not properly managed [12]. However, the N-urea and several other minerals present can serve as a source of nutrients in agriculture for growth with soil or without soil. Cow urine also contains Na, Ca, Fe, Mg, Al, K, and Zn [13], which are macro- and micro-nutrients required for plant development. Several studies revealed that its presence in various forms of liquid fertilizer formulations, which were applied through the leaves improved the growth performance and yield of plants cultivated on a soil [14]. [15] also reported that spraying a mixture of 25% liquid from cow bio slurry and 75% AB mix mineral fertilizer solution can increase the quality of corn fodder cultivated through a hydroponic system.

Seaweed (*Kappapychus alvarezii*) is one of the *primadonna* commodities in the fisheries sector of the Southeast Sulawesi government, hence, its cultivation areas can be found along the coastal waters of the province. Apart from carrageenan production, its biomass can also store some amount of nutrients and growth regulators, which are very useful to crops in various cultivation systems [16,17]. Seaweed extract contains some plant growth-promoting substances, such as Indole 3-acetic acid (IAA), gibberellins (GA₃), kinetin, zeatin [18,19], as well as macro- and micro-nutrients, including N, P, K, Na, Ca, Mg, S, Cu, Fe, Mn, and Zn [20]. The application of its liquid extract through leaves improved seed germination performance, growth, yield, and nutrient uptake. This has led to an increase in the use of chemical fertilizers in soil-based crop cultivation systems [21,22]. Therefore, this study aims to determine (i) the effect of replacing commercial nutrient solutions with liquid extracts of seaweed and cow urine on the growth of lettuce in a hydroponic system, and (ii) the amount of the commercial nutrient solution that can be substituted by the liquid seaweed extract and cow urine solution.

2 Material and method

This study was carried out from December 2020 to March 2021 in the Greenhouse of the Faculty of Agriculture, Halu Oleo University, which is located at 4°00.75'096' latitude, 122°31.55'01' east longitude, and 13 m above sea level. The treatment solution consists of a combination of AB mix solution (LHK), seaweed liquid extract (ECRL), and cow urine (US).

Subsequently, eight treatment combinations were tested, such as listed in the Table 1. They were then repeated three times and arranged in a randomized block design.

Table 1. Treatments were tested.

Symbols	Treatments
N0	100% LHK + 0% ECRL + 0% US
N1	50% LHK + 25% ECRL + 25% US
N2	50% LHK + 50% ECRL + 0% US
N3	50% LHK + 0% ECRL + 50% US
N4	25% LHK + 75% ECRL + 0% US
N5	25% LHK + 50% ECRL + 25% US
N6	25% LHK + 25% ECRL + 50% US
N7	25% LHK + 0% ECRL + 75% US

The AB mix mineral fertilizer was purchased from a farm shop. A total of 1,250 g fertilizer A was dissolved in 5 L of water, and it was stored as stock solution A. The same procedure was also carried out for fertilizer B, and it was labeled as stock nutrient solution B. Fresh seaweed (*K. alvarezii*) aged 35 days was washed with tap water, cut into sizes of 2-3 cm, and crushed using a kitchen blender until a slurry was obtained. It was then filtered using a cotton cloth, and the sap droplets were stored as a stock solution with 100% concentration. Subsequently, urine was mixed with feces excreted by cows in a 10 L bucket every morning for three consecutive days. For each collection, it was separated from the dung immediately with a fluor filter in the kitchen. The liquid in the plastic tray was then stirred daily for 14 days, after which the urine was filtered with a Whatman No. 1 filter paper and stored until use [23].

The N0 treatment was prepared by adding 12 mL of stock solution A to 12 mL of solution B. Subsequently, three liters of tap water was added to the mixture and stirred until it was homogeneous. For the N1 treatment, 12 mL of stock solutions A and B were mixed, followed by the addition of water to a total volume of 1,500 mL. 750 ml of 25% ECRL and 750 mL of 25% US were then added to the mixture. For N2 treatment, 12 mL stock solutions A and B were mixed, followed by the addition of water to a total volume of 1,500mL. Furthermore, 1,500 mL of 50% ECRL and 0 mL of 0% US were added. The same method was then used to prepare the remaining treatment solutions.

The plastic tray was filled with rice husk charcoal to a thickness of ± 3 cm, after which it was moistened using tap water. Lettuce seeds purchased from an agricultural store were then sown on its surface and placed in a dark room. After germination, the tray was placed in a room with enough direct sunlight. The humidity of the media was maintained by spraying an adequate amount of water. Subsequently, 2.5 mL of nutrient solutions A and B were dissolved in water to a volume of 1,000 mL. It was then sprayed through the leaves until the seedlings were ready to be transplanted into the growing media, which was prepared using a hydroponic installation.

A 25 cm x 25 cm nutrition tube was produced from used mica and then covered with black paint all over the outer surface. Several holes with a diameter of 7cm were bored on the top with a distancing of 4 cm. Meanwhile, the net pots were made of used plastic cups, and holes were perforated on the sides for root aeration as well as the bottom for placing the wick of the 25cm long flannel that connects the roots and nutrient solution. The mixture of biochar and fine sago dregs (1:1) was then placed into the pots up to its volume. Lettuce seedlings aged 10 days with 3-4 leaves were transplanted into each of them, placed in a hydroponic installation filled with treatment nutrient solution, and maintained until harvest.

The plant height and the number of leaves were observed at 7, 14, 21, 28, 35, and 42 days after planting (DAP), while the fresh and dry weight were measured at 45 DAP. The height was measured using a ruler with a maximum size of 30 cm, from the base to the tip of the

longest leaf. The leaf blades that were fully opened were also counted. The fresh weight was then determined by taking plants from each net pot without damaging the roots, after which the remaining growth media and roots were removed by hand. The samples were then weighed using an analytical balance with a maximum capacity of 1,000 g. They were placed in a sample paper, dried in an oven at 45°C to a constant weight, and weighed again using an analytical balance with the same capacity.

Analysis of variance for each plant growth parameter was carried out using the Fisher's exact test (F_{test}). When the value of $F_{\text{calculated}}$ is greater than that of F_{table} at $p < 0.05$, the relevant data were further analyzed with the High Significant Difference (HSD) test at $p < 0.05$ to determine the difference between the treatments.

3 Results and discussion

Equations should be centered Analysis of variance showed that the combination of mineral solution AB mix, seaweed liquid extract, and cow urine only had a significant effect ($F_{\text{calculated}} > F_{\text{table}}$ at the $p < 0.05$ level) on the plant height at 7, 14, and 42 DAP, as shown in Table 2. They also had a significant effect on the number of leaves at 35 DAP ($F_{\text{calculated}} > F_{\text{table}}$ at the $p < 0.05$ level), while it was not significant at other times ($F_{\text{hit}} < F_{\text{tab}}$ at $p > 0.05$). Furthermore, the nutrient solution mixture had a significant effect ($F_{\text{calculated}} > F_{\text{table}}$ at the $p < 0.05$ level) on the fresh and dry weight of the plants at harvest.

Table 2. Variance analysis of the effects of combining AB mix solution, seaweed liquid extract, and cow urine on lettuce plant growth in a hydroponic system.

Variable	$F_{\text{calculated}}$	F_{table} (df _{treatment} = 7; df _{error} = 14) at the $p < 0.05$ level
Plant height 7 DAP	61.96 [*]	2.76
Plant height 14 DAP	6.24 [*]	2.76
Plant height 21 DAP	1.41 ^{ns}	2.76
Plant height 28 DAP	2.15 ^{ns}	2.76
Plant height 35 DAP	2.68 ^{ns}	2.76
Plant height 42 DAP	4.07 [*]	2.76
Leaves number 7 DAP	1.00 ^{ns}	2.76
Leaves number 14 DAP	1.26 ^{ns}	2.76
Leaves number 21 DAP	1.77 ^{ns}	2.76
Leaves number 28 DAP	1.60 ^{ns}	2.76
Leaves number 35 DAP	2.96 [*]	2.76
Leaves number 42 DAP	2.18 ^{ns}	2.76
Wet weight	10.01 ^{**}	2.76
Dry weight	10.01 ^{**}	2.76

Remarks: The number followed by the superscript* indicates the treatment has a significant effect, and the superscript ns shows the treatment has no significant effect at the $p < 0.05$ level.

The analysis of variance showed that the treatment had a significant effect on the height at 7, 14, and 42 DAP as well as the number of lettuce leaves, as shown in Table 2. The pattern of temporal effect on plant height was similar to that of Ahmed et al. (2021). Table 3 shows that lettuce seedlings grown in nutrient solution produced from a mixture of 50% AB mix, 25% seaweed extract, and 25% volume of cow urine (N1) was the tallest with a length of 3.23cm at 7 DAP. This result was significantly different from treatments N2, N3, N4, N5, N6, and N7, except N0. At 14 DAP, the tallest plant of 6.28 cm was obtained in N1, and this was significantly different from N2 and N3, but not significantly from N0, N4, N5, N6, and

N7. Furthermore, at 42 DAP, N1 had largest height of 12.49 cm, which was significantly different from N4 and N5, but not significantly from others.

Table 3. Plant height period (cm) of lettuce in mixed treatment of AB mix + seaweed extract + cow urine in a hydroponic system.

Symbols of treatment	Days After Planting (DAP)		
	7	14	42
N0	3.02 ± 0.06 ^d	5.91 ± 0.25 ^{bc}	10.65 ± 0.65 ^{ab}
N1	3.23 ± 0.06^d	6.28 ± 0.25^c	12.49 ± 0.65^b
N2	2.35 ± 0.06 ^{bc}	4.83 ± 0.25 ^{ab}	10.35 ± 0.65 ^{ab}
N3	2.65 ± 0.06 ^c	4.25 ± 0.25 ^a	10.83 ± 0.65 ^{ab}
N4	2.06 ± 0.06 ^{ab}	5.27 ± 0.25 ^{abc}	8.68 ± 0.65 ^a
N5	1.87 ± 0.06 ^a	5.56 ± 0.25 ^{bc}	8.49 ± 0.65 ^a
N6	2.08 ± 0.06 ^{ab}	5.20 ± 0.25 ^{abc}	9.17 ± 0.65 ^{ab}
N7	2.18 ± 0.06 ^b	5.27 ± 0.25 ^{abc}	9.73 ± 0.65 ^{ab}
HSD (p=0.05)	0.31	1.25	3.27

Remarks: The numbers (mean ± s.e, n = 3) followed by different letters in the same column show significant differences according to the HSD test at the level of $p < 0.05$.

The different plant height in the treatments indicated that there were differences in nutrient availability in each component of the solution mixture. However, there was no significant difference between treatments N1 and N0, which shows that the nutrients and phytohormones in the mixture of seaweed extract and cow urine can replace the growth function of the 50% AB mix volume, which was removed [21,24]. These results also implies that the concentration of growth-promoting substances was in the appropriate range to promote the development of the shoots and roots. The growth of roots increases the capacity of the plants to absorb macro- and micro-nutrients from the solution to the leaf [25].

The effect of the treatment on the number of lettuce leaves was only significant at 35 DAP, as shown in Table 2. This pattern of timing influence was assumed to be related to the synchronization between nutrient availability, the phase of maximum root capacity to absorb nutrients as well as the need for lettuce leaf formation. Some parts of the plant respond to availability when certain levels of nutrients needed for their growth plant are within the critical limits [26]. The capacity of the growing medium to supply the micronutrients needed for the formation of new leaves, such as N, P, K, Ca, and Mg, is majorly influenced by environmental conditions [27, 28]. Furthermore, pH and electrical conductivity (EC) are important indicators for regulating availability as well as the capacity of lettuce roots to absorb nutrients in hydroponic systems [29]. Low pH levels can cause abiotic stress in the root zone and restrictions on phosphorus availability [30]. A high EC value can harm the lettuce plant growth due to osmotic stress, while low levels indicate an inadequate nutrient mineral content in the growing solution, which limits the development of certain parts [31]. The value of pH and EC varied during the growth period in the hydroponic system [32]. In terms of pH changes, the supplement prepared from organic mineral and mineral solution was still within the appropriate range for development in the tropics [33]. The EC value showed that the capacity of the organic mixture to supply nutrients decreased drastically compared to the inorganic variants. The treatment tested in this study aims to modify the standard mineral nutrient plant solution or AB mix, which is commonly used in lettuce cultivation. Modifications were carried out by reducing its volume, while increasing the supplements, namely seaweed extract, cow urine or their mixture. These additives are known as liquid organic fertilizer solutions [34]. These findings help to provide a possible explanation for the apparent effect of the treatment on lettuce leaf count at 35 DAP.

Figure 1 reveals that treatment N0 had the highest number of leaves, namely 7.42 leaves/plant at 35 DAP, which was significantly different from others. This indicates that the number of lettuces grown in the standard medium was higher than the modified variant. Based on the results, the number of nutrients obtained from *K. alvarezii* extract, cow urine their mixture cannot replace the reduction in volume of the growing solution. [35] reported that the number of lettuce leaves grown with organic growth solution prepared from molasses fermentation and sugarcane leaves were lower than the inorganic nutrient variant. In terms of plant height, the highest value was obtained from treatment N1 containing 50% AB mix + 25% seaweed extract + 25% cow urine. This indicates that lettuce tends to grow larger than in the supplementation compared to 100% AB mix solution (N0), while the number of leaves is the opposite. These results provide an illustration that each part of the plant responds differently to stimuli from the influential growth environmental factors.

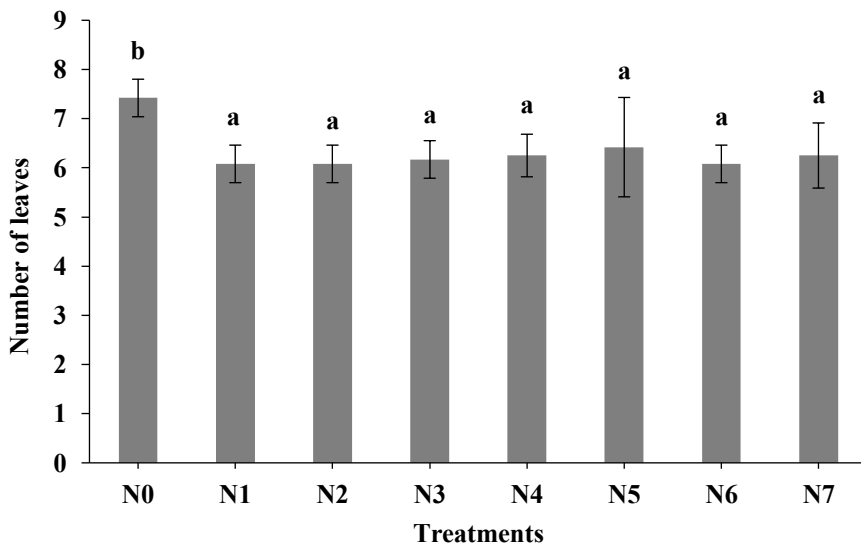


Fig. 1. Differences in the number of leaves of lettuce between treatments at 35 days after planting. The different letters above the bar show the significant differences based on the HSD test at the $p < 0.05$ level.

Farmers often sell their lettuce harvest in the form of fresh biomass. Significant differences were observed in the fresh and dry weight obtained in the various treatments, as shown in Table 4. The newly harvested plants in N1 were the heaviest with an average weight of 42.72 g/plant, and this was significantly different compared to others, except N0. The lightest produce with an average weight of 30.15 g/plant was recorded in N7, which was only significantly different compared to N0 and N1, as shown in Table 4. This difference was assumed to be related to the amount of available nitrogen as well as the rate of uptake by the roots in the growing solution [36].

Table 4 shows that the highest dry weight of 1.51 g/plant was recorded in N1, and this was significantly different from other treatments, except N0. Meanwhile, N7 had the lightest of 1.06 g/plant, which was significantly different from only N0 and N1. The increase in fresh and dry weight was caused by the macro and micro-nutrient content as well as the concentration of phytohormones in the growing medium solution. The N1 treatment was suitable for triggering root growth, improving respiration, mobilization, and mineralization of nutrients, increasing nutrient uptake, and enhancing the formation of plant biomass [37,38].

Table 4. Differences in fresh and dry weight of lettuce between the treatment of a mixture of inorganic nutrient solution + seaweed extract + cow urine in a hydroponic system.

Treatments	Wet weight (g)	Dry weight (g)
N0	40.09 ± 1.37 ^{bc}	1.41 ± 0.05 ^{ef}
N1	42.72 ± 1.37^c	1.51 ± 0.05^f
N2	33.53 ± 1.37 ^{ab}	1.18 ± 0.05 ^{de}
N3	34.48 ± 1.37 ^{ab}	1.22 ± 0.05 ^{de}
N4	32.92 ± 1.37 ^a	1.16 ± 0.05 ^d
N5	31.18 ± 1.37 ^a	1.10 ± 0.05 ^d
N6	33.91 ± 1.37 ^{ab}	1.20 ± 0.05 ^{de}
N7	30.15 ± 1.37 ^a	1.06 ± 0.05 ^d
HSD (p=0.05)	6.83	0.24

Remarks: Numbers (mean ± s.e. n = 3) followed by different letters in the same column show significant differences according to the HSD test at the $p < 0.05$ level.

4 Conclusion

The growing solution from a mixture of cow urine and seaweed liquid extract has the potential to minimize the use of nutrient solutions from inorganic fertilizers, namely AB mix to grow lettuce plants through a hydroponic system. Approximately by 50% volume of inorganic variant can be reduced through the application of a mixture of urine and extract in a 25% proportion of the total volume. Future studies need to be directed to the economic assessment of this growing solution mixture in hydroponic cultivation systems.

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