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RESEARCH PAPER

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EFFECTS OF DIFFERENT ORGANIC FERTILIZER APPLICATIONS IN SOILLESS LETTUCE CULTIVATION

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ABSTRACT

The present study was conducted for the purpose of investigating the potential of different organic fertilizers as alternatives to chemical fertilizers. Three organic fertilizers (protein hydrolysate – PH, black soldier fly manure – BSF and wood ash – WA) were compared with a chemical fertilizer (15-15-15+ME) as a control. The fertilizers were applied at a rate of 1.5 dS m^{-1} once a week, at a rate of 1 L $plant^{-1}$. The results show that the highest values for plant height (23.9 cm), leaf number (24.7), crown diameter (35.2 cm), photosynthesis rate (83.2 µmol $m^{-2}s^{-1}$), plant dry weight (16.2 g) and yield (324.8 g $plant^{-1}$) were observed in lettuce plants treated with PH organic fertilizer. The highest leaf chlorophyll content (8.6 CCI) and stomatal conductance (408.7 mmol $m^{-2}s^{-1}$) were recorded in the control, while BSF manure significantly increased root length (36.8 cm) and root dry weight (6.2 g). The potential of organic fertilizers as a viable alternative to chemical fertilizers in the soilless lettuce cultivation has been demonstrated. The utilization of protein hydrolysate has emerged as a pivotal aspect in lettuce cultivation. The nitrogen content of organic fertilizers has been identified as a plant nutrient element affecting success in lettuce cultivation.

Keywords: black soldier fly manure, chemical fertilizer, protein hydrolysate, wood ash

INTRODUCTION

The engagement in agricultural activities is driven by the objective of meeting the population's dietary needs. This socio-economically significant issue has resulted in the emergence of systems in which chemical fertilizers are employed as an alternative to traditional agricultural methods, in parallel with the global population increase. The widespread and indiscriminate use of chemical fertilizers has exerted deleterious effects on soil quality and human health, thus necessitating the exploration of alternative cultivation systems, such as soilless farming. Soilless cultivation has become increasingly common, particularly for fast-growing vegetables such as lettuce, with the objective of enhancing both yield and quality [Savci 2012, Şahin 2016, Saraçoğlu et al. 2022, Durak and Polat 2025].

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Among leafy vegetables, lettuce (*Lactuca sativa* L.) is the most widely cultivated crop, and our country holds a significant position in global lettuce production. Lettuce, generally consumed fresh, is a vegetable rich in minerals, particularly from a nutritional standpoint [Okudur and Ercan 2016, Kilim et al. 2022]. Lettuce is categorized as a cool-season vegetable, showing strong resistance to low temperatures and requiring high humidity. Due to its short vegetation period, this species can be cultivated in all regions of Turkey and possesses considerable economic value [Yıldırım et al. 2015, Kilim et al. 2022].

Soilless agriculture is a cultivation method that is particularly effective in terms of the efficient use of limited agricultural land. It is also posited that the practice has the capacity to reduce environmental risks through the regulated utilization of nutrients. Nevertheless, even within these systems, a reliance on chemical fertilizers remains entrenched. Consequently, there is a compelling need to explore the utilization of alternative organic and biological materials as fertilizers [Durak and Polat 2025].

The present study examined the feasibility of utilizing various organic fertilizers as substitutes in soilless lettuce cultivation. The primary motivation for the research is to reduce the use of chemical fertilizers by promoting plant growth through the utilization of various organic fertilizers, either individually or in combination with chemical fertilizers. To this end, an experiment was conducted to examine the effects of three different organic fertilizers (protein hydrolysate, insect fertilizer, and wood ash) on soilless lettuce cultivation.

Materials and methods

The study was conducted at Ondokuz Mayıs University, Faculty of Agriculture, Department of Horticulture, between December 20, 2024, and February 12, 2025. The study was conducted in a greenhouse (120 m^2) using fully automated soilless cultivation. Two climate control fans were installed in the greenhouse to maintain the humidity and temperature during cultivation. Lettuce seedlings (*Lactuca sativa* var. *crispa* Maritima) were planted in the growing pots, which were placed 25 cm apart in rows. The lettuce seedlings used in the study were obtained from a commercial company (Agtohum, Antalya, Turkey). The seedlings were planted in 7-liter plastic pots containing coconut coir (cocopeat) on a 5% sloped gutter, 40 cm above ground level for their growing environment on December 20, 2024.

The process of fertilization was initiated at the moment of sunrise and concluded at sunset, during the period of the growing season. The irrigation schedule was set to irrigate every 30 minutes for 2 minutes each time. Additional fertilizers were applied manually once a week according to the procedure specified in the experimental setup.

Climate data were recorded using data loggers (Hobo, MX2301A and H21 Onset, Bourne, USA) during the greenhouse cultivation period. Temperature values were measured at an average of 19.8 °C. Average relative humidity values were determined to be 40.8%. Measured average light values were 186.2 μmol $m^{-2}s^{-1}$ (PAR).

Experimental setup

For chemical fertilization (control), the compound chemical fertilizer 15–15–15+ME (Gübretaş, Istanbul) was used. The fertilizer's composition is reported as total nitrogen (15%), ammonium nitrogen (9%), urea nitrogen (6%), phosphorus pentoxide (22%), neutral ammonium citrate and phosphorus pentoxide (25%), potassium oxide (10%), sulfur trioxide (10%), and zinc (0.5%).

Table 1. Nutrient content of organic fertilizers

Elements	Protein hydrolysate (PH)	Black soldier fly manure (BSF)	Wood ash (WA)
EC (dS m^{-1})	2.39	2.92	13.6
pH	5.56	8.04	12.2
N (%)	10.5	3.2	0.13
P (ppm)	8808.3	650	7826
K (ppm)	23500	17000	66742
Ca (ppm)	214.1	13.9	66200
Mg (ppm)	454.1	0.4	43000
Zn (ppm)	60	166.6	36
Mn (ppm)	3.3	164.5	20000
Cu (ppm)	3.6	40.3	60
Fe (ppm)	82.5	841.2	1850

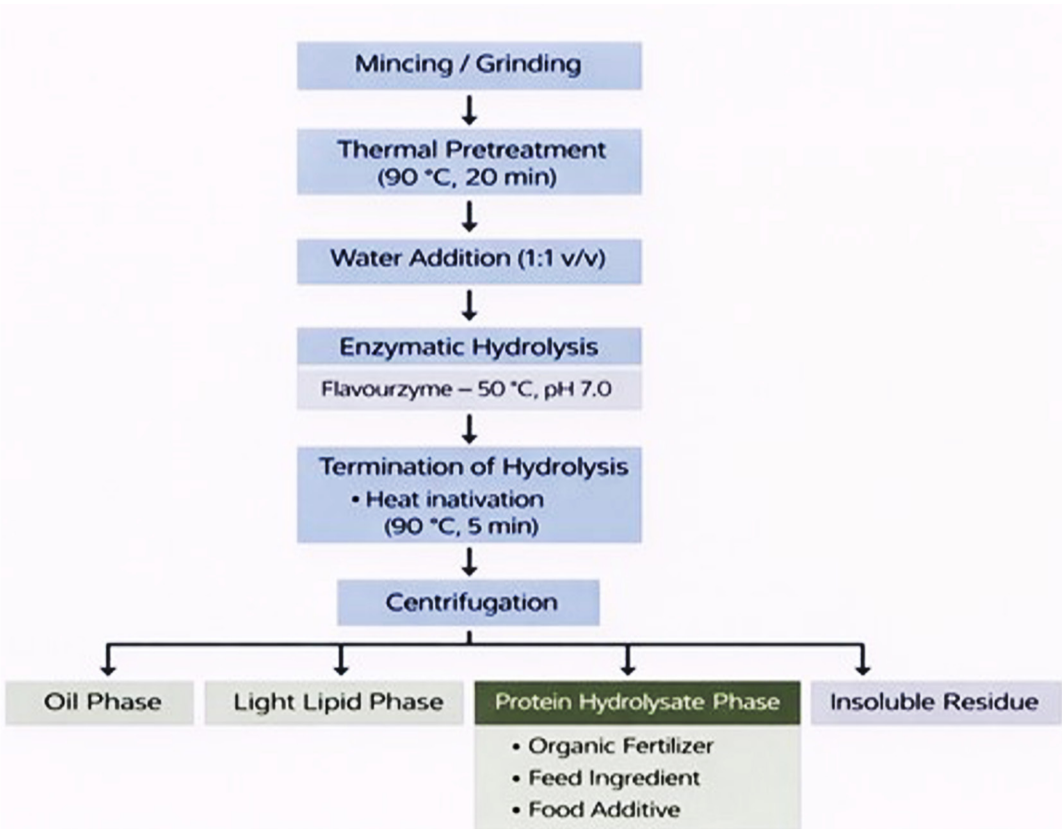
The EC value of the fertilization solution was set at 1.5 dS m^{-1}, in accordance with the findings of Kilim et al. [2022] and Çilingir Tütüncü et al. [2025]. In order to achieve an EC value of 1.5 dS m^{-1} for chemical fertilization, it was necessary to dissolve 2.27 g of fertilizer in 1 L of water. The study utilised three distinct organic fertilizers (protein hydrolysate – PH, black soldier fly manure – BSF, and wood ash – WA), and a chemical fertilizer for the purpose of establishing a control group. The analyses of the obtained fertilizers (PH, BSF, WA) solutions were made according to Kacar and İnal [2008] and are given in Table 1.

Preparation of organic fertilizers

The preparation steps for the organic fertilizers, whose plant nutrient content is detailed above, are described in detail below.

Protein hydrolysate (PH). In the study, anchovy (*Engraulis encrasicolus*) processing waste was obtained from SASTAŞ (Samsun Soğutma Tesisleri A.Ş https://www.sastas.com.tr). SASTAŞ processes fresh and frozen seafood in accordance with HACCP, BRC Food, and IFS Food Safety Standards, Social (Ethical) Standards, and National and International Legislation. The waste obtained from the facility was vacuum-packed in 5 kg packages, placed in foam boxes filled with ice, and transported to the Processing Technology Department laboratory of the Department of Fisheries Technology Engineering, Faculty of Marine Sciences, Fatsa, Ordu University, where it was stored at –40 °C until protein hydrolysate was obtained. The waste was thawed at room temperature using a mincer, then minced and homogenized. The material was subsequently heated at 90 °C for 20 minutes to ensure the inactivation of endogenous enzymes. The minced fish meat, with its enzymes inactivated, was cooled and homogenized by adding pure water at a 1 : 1 ratio. Protein hydrolysate from fish waste was obtained by enzymatic hydrolysis (Figure 1), following the method used by international companies, according to Korkmaz [2018]. When preparing the protein hydrolysate solution, 1 g of PH was added to 1 liter of water to achieve an EC of 1.5 dS m^{-1}.

Figure 1. Process flow diagram of fish protein hydrolysate production from anchovy (*Engraulis encrasicolus*) processing by-products



Black soldier fly manure (BSF). For the purposes of this study, a mixture of waste weighing 50 kilograms was prepared. The mixture was prepared by blending 30% (15 kg) of fish waste, 40% (20 kg) of vegetable and fruit waste, and the remaining 30% (15 kg) of household organic waste. A total of ten plastic containers, each measuring 64 × 40 × 10 cm, were utilised for the purpose of storing the mixed and homogenised waste, with each container containing 5 kg of the aforementioned waste. The experiment involved the use of 50 grams of 5-day-old black soldier fly (*Hermetia illucens*) larvae, which had been freshly hatched from eggs, and these were then weighed and placed into each container. A feeding study was conducted for 20 days until the larvae pupated. Subsequently, the pupae were extracted from the plastic containers, and the residual larval frass was subjected to desiccation and conversion into fertilizer.

Wood ash (WA). Waste ash of oak (*Quercus* sp.) species was used to prepare the wood ash solution. Waste ash was taken from a bakery shop in Samsun province (Yazıcıoğlu Kardeşler Bread Bakery). A solution with an electrical conductivity of 1.5 dS m^{-1} was prepared from wood ash. While creating the solution, 11 g of wood ash was added to 1 liter of water.

Fertilization for all treatments began four days after planting, on December 24, 2024, and was applied weekly at a rate of 1 liter per application. The study concluded on February 12, 2025, the harvest date.

Plant measurements

On the designated harvest date, the stem and crown diameters of the measurement and observation plants were measured using a digital caliper. The number of leaves was also counted. Leaf chlorophyll content (CCI), stomatal conductance (mmol $m^{-2}s^{-1}$), and photosynthesis rate (μmol $m^{-2}s^{-1}$) were measured between 9.00 and 11.00 a.m. on leaves at different levels of the measurement plants (3 leaves per plant). Leaf chlorophyll content was measured using a CCM-200 chlorophyll meter (Opti-Sciences, USA). The assessment of stomatal conductance was performed employing an SC-1 leaf porometer (Decagon Devices, Pullman, USA). The quantitation of photosynthesis rate values was accomplished using a Mini PPM (EARS, Wageningen, Netherlands) device.

Lettuce plants were extracted from the soil and divided into roots and leaves. The rosette mass was determined by weighing the fresh weight of each plant. Roots were washed with tap water to remove all soil particles. The root length was measured in centimeters. The leaves and roots were then placed separately in small paper bags and dried in an oven (Venticell 55, Ecocell, MMM Group, Germany) at 80 °C for 48 hours. The weight change method was used to determine when the drying process was complete. The dried leaf and root samples were weighed using a digital scale.

Statistical analysis

In this study, which was established according to a randomized block design, measurements were taken on a total of 60 plants, with 4 treatments, 3 replications, and 5 measurement and observation of plants in each replication. Differences between groups were determined using Duncan's multiple comparison test ($p > 0.05$). The OMU-licensed SPSS 21 software package was used for data analysis.

Results and discussion

The seedlings of the lettuce *Lactuca sativa* var. *crispa* Maritima (planted in four true-leaf stages), were ready for harvest 54 days after planting. Harvest times in lettuce cultivation vary from 36 to 80 days. Temperature has a significant effect on the harvest date. It has been reported that lettuce grown without soil is harvested earlier than lettuce grown in soil [Maboko and Du Plooy 2007, Okudur and Ercan 2016, Yörük et al. 2021, Kilim et al. 2022].

In our study, different fertilizer (PH, BSF, WA and Control) applications were found to have significant effects ($p < 0.05$) on the plant height, leaf number, crown diameter, chlorophyll content, stomatal conductance, and photosynthesis rate of lettuce grown in a soilless cultivation.

According to the results, the highest plant height (23.9 cm), number of leaves (24.7), crown diameter (35.2 cm), and photosynthesis rate (83.2 μmol $m^{-2}s^{-1}$) were measured with the PH organic fertilizer application, while the highest chlorophyll content (8.6 mmol $m^{-2}s^{-1}$) and stomatal conductance (408.7 mmol $m^{-2}s^{-1}$) were measured with the control application. However, for leaf chlorophyll content, crown diameter, and leaf number values, BSF manure and control applications were similar and measured lower values, while photosynthesis rate values were measured similarly for BSF manure. The WA treatment yielded the lowest values in general (Table 2). It is hypothesized that the low nitrogen content of the WA organic fertilizer may have been a contributing factor (Table 1).

Table 2. The effects of different organic (PH, BSF and WA) and chemical (control) fertilizers on plant height, leaf number, crown diameter, leaf chlorophyll content, stomatal conductance and photosynthesis rate in soilless lettuce cultivation

Fertilizers	Plant height (cm)	Leaf number	Crown diameter (cm)	Chlorophyll content (CCI)	Stomatal conductance (mmol $m^{-2}s^{-1}$)	Photosynthesis rate (µmol $m^{-2}s^{-1}$)
Control	20.8 ±1.84b	23.7 ±1.56a	33.5 ±2.56a	8.6 ±1.11a	408.7 ±6.80a	76.4 ±3.61b
PH	23.9 ±1.61a	24.7 ±1.04a	35.2 ±1.04a	7.3 ±1.18ab	310.8 ±6.05b	83.2 ±6.54a
BSF	22.2 ±0.54ab	24.2 ±1.61a	33.8 ±1.61a	8.5 ±1.16a	282.0 ±8.81b	80.8 ±5.87a
WA	9.8 ±0.57c	16.5 ±1.32b	17.3 ±1.32b	6.9 ±0.31b	189.7 ±4.11c	74.0 ±3.56b

Notes: means in the same column followed by different letters were significantly different ($p < 0.05$)

Plant-derived protein hydrolysates (PH) are attracting global attention due to their sustainability and positive effects on plants under abiotic stresses. The effects of these known effects on yield and quality in lettuce and tomato cultivation were investigated. For this purpose, PH (0 or 3 g L^{-1}) was applied to plants fertilized with four different N levels (2, 5, 10, and 15 mM) via foliar and root application. In the study, root application effectively stimulated yield, photosynthesis, water use efficiency, chlorophyll content, and antioxidant activities and compounds at all nitrogen levels compared to foliar application [Choi et al. 2022]. According to Cilingir Tuttuncu et al. [2025] compost solution, nettle solution, and wood ash solution have been identified as important alternatives to chemical fertilizers in lettuce cultivation. Wood ash applications have been reported to be notable for plant growth parameters. When lettuce was grown in soil, the application of fermented plant water, fermented fruit juice, and commercial organic leaf fertilizer had significant effects on yield and aroma, particularly on plant height (6.48–11.06 cm) and root length (4.19–7.26 cm), compared to the control [Caberoy and Ortuoste 2025]. Earthworm castings alone or in combination with different chemical fertilizers have been found to have significant effects on lettuce growth performance. While the best results were obtained with 100% chemical fertilizer application, 50% control + 20 ml/L earthworm castings-cow manure performed better than all other combinations. Specifically, this was attributed to leaf width, root length, root volume, root weight, leaf weight per plant, and total yield results [Ramos 2026]. The findings of our study are consistent with other studies on soil-less lettuce cultivation [Aruho et al. 2026, Bantis et al. 2026, Ghimire et al. 2026, Parra et al. 2026].

Our study determined that different fertilizers (PH, BSF, WA, and control) had significant ($p < 0.05$) effects on yield, dry weight, root length, and root dry weight. In our study, the highest yield (324.8 g $plant^{-1}$) and dry weight were obtained from the application of PH organic fertilizer, while the highest root length (36.8 cm) and root dry weight (6.2 g) were obtained from the application of BSF manure (Table 3).

Table 3. The effects of different organic (PH, BSF and WA) and chemical (control) fertilizers on yield, dry weight, root length and root dry weight in soilless lettuce cultivation

Fertilizers	Yield (g $plant^{-1}$)	Dry weight (g $plant^{-1}$)	Root length (cm)	Root dry weight (g)
Control	244.5 ±7.16b	14.7 ±0.71b	35.2 ±3.65a	3.7 ±0.78bc
PH	324.8 ±5.33a	16.2 ±1.27a	32.2 ±2.02b	4.3 ±1.05b
BSF	297.7 ±4.41ab	12.0 ±0.18c	36.8 ±2.88a	6.2 ±0.69a
WA	86.7 ±6.53c	8.7 ±0.35d	36.4 ±2.69a	3.1 ±0.57c

Notes: means in the same column followed by different letters were significantly different ($p < 0.05$)

The study found that root application effectively stimulated yield, photosynthesis, water use efficiency, chlorophyll content, and antioxidant activities and compounds at all nitrogen levels compared to foliar application. PH applications significantly increased fresh weight (21%) and dry weight (31%) in lettuce [Choi et al. 2022]. In the study of Çilingir Tütüncü et al. [2025], three different organic fertilizers (compost solution, nettle solution, and wood ash solution) were tested in lettuce cultivation as alternatives to chemical fertilization. The highest yield was obtained from wood ash solution applications (555 g plant^{-1}), while the lowest yield was measured in chemical fertilizer applications at 402 g plant^{-1}. Unlike our study, this study obtained the highest yield from wood ash appli-cation. We believe this situation stems from the high organic matter content (4.36%) of the soil in which the study was conducted. This is because nitrogen is known to be the key nutrient determining success in lettuce cultivation [Choi et al. 2022, 2025]. To this end, the effectiveness of using nitrogen in combination with PH was investigated. Specifically, the application of PH to the roots increased the yield (shoot fresh weight), quality (chlorophylls, ca-rotenoids, flavonoids, and phenols), and nutrient uptake in hydroponically grown lettuce [Choi et al. 2025]. The findings of these studies are consistent with the PH yield values in our findings.

In the study of Turan et al. [2026], six different biostimulants were investigated in lettuce cultivation, including combinations with no fertilizer, chemical fertilizer, and positive control. Total chlorophyll levels increased with biostimulant application, indicating that biostimulants enhance photosynthetic efficiency. Biostimulant applications significantly improved plant and stem diameter, fresh and dry biomass, and yield ($p < 0.01$). The best yield and morphological performance were obtained in samples receiving biostimulant-fertilizer combinations (Kiana Climate® + 75 : 50 : 75 kg ha^{-1} N : P : K) and T7 (Kiana Earth® + 150 : 100 : 150 kg ha^{-1} N : P : K) applications.

In soilless systems, composting and vermicomposting are identified as the primary methods that can be used in for production of biological or organic fertilizers. However, agricultural waste, kitchen waste, biological solids, rumen waste, slaughterhouse waste, market waste, plant leaves, and wastewater are also available. Nutrients, compost teas, vermicompost teas, fermentation (immersion, solid state, co-culture, bokashi), and algae tea can be obtained from these substrates. It is emphasized that the development of the most suitable liquid biological fertilizer formulations depends on the optimal level of nutrients and the presence of beneficial microorganisms being higher than that of harmful pathogens [Zirebwa et al. 2026]. Similar to this study, our study has shown that the nutrient content of PH and BSF organic fertilizers can be an important alternative in soilless lettuce cultivation.

CONCLUSION

Thanks to its many cultivation and marketing advantages, lettuce can be grown year-round using soilless methods. In soilless lettuce cultivation, the use of fertilizers can be reduced by using natural and sustainable resources. Furthermore, this approach can have significant environmental impacts through improved waste management. Considering all these characteristics, it has been demonstrated that PH and BSF manure applications in soilless lettuce cultivation can serve as alternatives to chemical fertilization. The main factor limiting WA fertilizer is the nitrogen content of the waste. However, we believe that the high potassium and phosphorus content of WA waste will increase efficiency when used in combination with other organic fertilizers.

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Not applicable.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

DECLARATION OF THE USE OF genAI TOOLS IN THE PROCESS OF PREPARING THE PAPER

Deepl AI was employed as an assistive proofreading instrument for the article.

AUTHORS CONTRIBUTIONS

A.Ç.T, A.Ç.: investigation, data curation and visualization. S.D.: plant nutrient analysis, writing, investigation, data curation, and visualization. İ.C.D., O.Y.: preparation of black soldier fly fertilizer, investigation, writing, editing. K.K.: preparation of protein hydrolysate fertilizer, investigation, writing, editing. H.Ö.: writing, reviewing, supervision, investigation, methodology, conceptualization, data curation, project administration.

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